



## **Communicating spatial variation in tick-borne pathogen prevalence through a website based on national surveillance data**

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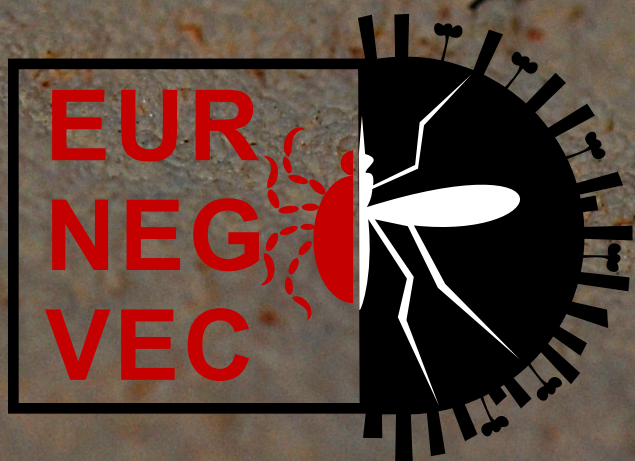


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**ABSTRACT BOOK**





## WG 1

# The “One Health” concept in the ecology of vector-borne diseases

## ORAL PRESENTATIONS

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## CANINE VECTOR BORNE DISEASES IN SHELTERS FROM THE BORDER REGIONS OF MAINLAND PORTUGAL

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Canine Vector Borne Diseases (CVBDs) are infectious diseases increasingly reported worldwide that represent a serious threat to both animal and public health. The Iberian Peninsula is considered an area of high endemicity for CVBDs, situation related to the presence of competent vectors and the mild Mediterranean climate that favours its development and survival. However, concise data on the epidemiological situation is scarce, particularly in the inland regions. To assess the current situation at the borders of Portugal with Spain, a large epidemiological survey was designed involving the blood collection from 248 apparently healthy domestic dogs, kept in shelters from seven districts of Portugal (Beja, Bragança, Castelo Branco, Évora, Faro, Guarda and Portalegre). Sera were tested by enzyme-linked immunosorbent assay (ELISA) for detection of specific antibodies to *Leishmania infantum*. Commercial immunofluorescent antibody tests (IFAT) MegaScreen® kits were used to detect the IgG-antibodies to *Rickettsia* spp. and *Babesia* spp. Commercial qualitative antigen kit WITNESS® *Dirofilaria* (Synbiotics, Europe) was used to test for the presence of *Dirofilaria immitis* circulating antigens. Additionally, Giemsa-stained blood smears were evaluated for the presence of hemoparasites and modified Knott's technique was performed for microfilariae identification and differentiation. Ninety-one percent of the dogs were seropositive for antibodies to *Rickettsia* spp., 8.6% to *Babesia* spp. and 7.7% to *L. infantum*. In total, 2.8% were infected by *D. immitis* (antigen and/or *D. immitis* microfilariae) and 1.2% by *Acanthocheilonema* spp. No *Dirofilaria repens* microfilariae were detected. Parasitic agents were observed in 44% of the blood smear samples, of which 36.3% were consistent with epierythrocytic bacteria typical of *Mycoplasma* spp., 9.7% with gamonts of *Hepatozoon* spp. and 6.9% with intraerythrocytic merozoites of *Babesia* spp. These results reveal a high occurrence and circulation of several CVBD agents in the border regions, many of them of

zoonotic concern. The lack of funding in animal shelters, regardless of all the volunteer human and economical effort, constitutes a real challenge in the implementation of regular prophylactic measures and disease management. The difficulty to eliminate the circulation of these pathogenic agents perpetuates these parasitic infections, compromising Animal Health and Welfare. Besides, this may constitute a potential Public Health menace for those involved in animal care and for future adoption programs. Data from this study highlight the urgent necessity to adopt strategies to prevent and control parasitic agents in dogs from Portugal and Spain.

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## **SEROPREVALENCE FOR WEST NILE VIRUS, *COXIELLA BURNETII* AND *BORRELIA BURGDORFERI* SENSU LATO IN A HIGH RISK POPULATION**

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Danube Delta is a paradise for birds, mosquitoes, arthropods and sheep. Chilia Veche is a small village from Danube Delta with 2132 citizens, exposed to reservoirs and vectors for different infections. The average of age is over 40 and most residents work in agriculture, livestock and fisheries. The aim of our study was to evaluate the seroprevalence for West Nile virus, *Coxiella burnetii* and *Borrelia burgdorferi* in a very exposed population. Data regarding such a population is lacking in Romania. We collected blood samples, during two days in July 2015 during the summer school of COST action TD13030 (EurNegVec) from all healthy adults who have agreed to participate in our study by signing the informed consent and filling our questionnaire regarding risk factors for the infections. We measured the antibody titer using ELISA test for: West Nile virus, *Coxiella burnetii* phase 1 and 2 and *Borrelia burgdorferi* sensu lato, and we confirmed West Nile with neutralization test and *Borrelia* with western blot test. We performed a statistical analysis regarding risk factors for different infections. There were 71 patients (3.5% of population), 47 women (66%), average age 53, 51 residents spent all their life in Chilia Veche, 40 of residents spent all the day in outdoor activities, 66 recognized that are bitten by mosquitoes and 6 recognized history of tick bite, 49 have water reservoirs in their backyards, 52 have backyard birds and 13 have horses. 10 residents have occupational risk for Q fever. One resident recognized medical history of meningitis and one a skin lesion that could have been Erythema migrans. Seroprevalence for West Nile was 7%, for *Coxiella burnetii* 27% (60% for residents with occupational risk and 21% for those without

occupational risk, 21% for women and 37% for men, statistically higher below age 52,  $p=0.04$ ) and for *Borrelia burgdorferi* sensu lato 7%. No correlation was found between history of tick bite and serological findings for *Borrelia*. Only correlation between West Nile positive serology was found with age more than 67 and living in Chilia Veche more than 80% of the life. In conclusion, seroprevalence for *Coxiella burnetii* is high in this region of the country, especially in young males; longer exposure to mosquitoes bite is correlated with positive serology for West Nile virus; Population from rural area is not aware about tick bite even if the seroprevalence for *Borrelia burgdorferi* is high.

## RESULTS OF WNV MONITORING PROGRAMME FOR 2015 IN SERBIA

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The WNV monitoring program for 2015 in Serbia, launched by Veterinary Directorate, was based on direct and indirect monitoring of the presence of WNV in nature. Indirect monitoring of virus presence was performed by serological testing of horses on the presence of anti-WNV IgM antibodies as the confirmation of acute infection. Direct monitoring was done by molecular testing of WNV presence in pooled mosquito's samples and in wild birds. Number of tested samples is defined at the level of each County of the Republic of Serbia in relation to the risks of WNV infection. Between June and September 2015 in Serbia, 3238 blood sera of horses were tested, and seroconversion (IgM antibodies) was detected in 17 (0.53%) horses. Positive serological responses were determined in June in 0.13%, in July in 0.12%, in August in 1.11%, and in September in 0.77% of tested horses. Out of 25 counties in Serbia, the number of counties in which the positive horses were detected was: one in June, one in July, five in August and four in September. In direct monitoring of virus presence, 956 pools of mosquitoes were tested from June to September 2015, and WNV was confirmed in 20 (2.09%) samples. The prevalence of WNV in mosquitoes has increased since the first positive findings in June (0.38%); 1.13% mosquito samples tested positive in July, 4.92% in August and 1.85% in September. Positive mosquito samples were detected in 6 counties: 8.7% positive out of tested samples was detected in South Backa, 5.63% in South Banat, 4.41% in Western Backa, 4.22% in North Backa, 2.99% in Srem and 2.86% in Central Banat County. Among 183 samples of found dead wild birds, WNV was detected in 2 (1.09%) cases - in a hooded crow found in city of Novi Sad in August and in a carrion crow found in town Pancevo in September.



Among 13 samples of hunted Eurasian magpies WNV was detected in 3 (23.08%) cases – hunted near towns Svilojevo and Novo Orahovo, Wester Backa County, in September. WNV was also detected in 3 (0.52%) out of 524 tested samples of pharyngeal swabs of live wild birds (hen harrier near village Elimir and at the territory of Belgrade). The main objective of the monitoring program was the early detection of the presence of WNV in a certain area, and consequently timely alerting of human health services and local governments in order to control the mosquito population and to inform the local communities. WNV monitoring program during 2015 was proved as very successful and meaningful, despite some technical problems.

This work is conducted within the project TR31084 funded by the Serbian Ministry of Education, Science and Technological development and by Ministry of Agriculture.

## A SURVEY OF CANINE LEISHMANIOSIS IN ALBANIA

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Zoonotic visceral leishmaniasis (ZVL) is an important public health problem in Albania. An effort to control this disease involves understanding the epidemiology of this infection in both humans and reservoir dogs. During the first half of 2015, 16 human cases of ZVL were reported to the Institute of Public Health in Tirana. Blood collection from household dogs of these human patients and from the dogs living in the same area, but not belonging to patients was conducted in June and July of 2015. Nineteen areas were surveyed in total. The sera of dogs were examined by ELISA to detect anti-*Leishmania* antibodies. For each case, a detailed questionnaire was completed. Bio-statistical and geo-spatial methods were used for statistical analysis. Out of 308 dog sera, 10 resulted seropositive (3.2%) (95%CI 1.54–5.80) for *Leishmania* infection. Most of the cases 2.6% (8/308) were from rural areas and only 0.65% (2/308) from urban settings. We found 1.3% of the cases in mountainous areas followed by hilly and plain areas with ~1% each, respectively. Clinical signs compatible with canine leishmaniasis such as dermatitis, alopecia, ulcers, onychogryphosis, pale mucosal membranes, ocular lesions, or weight loss were found in 3 dogs in two localities. In conclusion, this study indicates that *Leishmania* infection in dogs is present in several areas in Albania. Canine leishmaniosis represents a threat to human public health. It is therefore necessary to implement strategies for the surveillance and control of canine leishmaniosis which could reduce the incidence of leishmaniasis in humans.

## CONTROL OF LYME BORRELIOSIS: SHOOTING THE MESSENGER

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In order to develop sustainable approaches to minimize the risk of Lyme borreliosis, we aimed to identify environmental drivers which affect the enzootic cycles of ticks and *Borrelia burgdorferi* s.l. Although *Ixodes ricinus* is a generalist tick, we found that only a few abundant vertebrate species dominate its life cycle. Whereas roe deer act as its main propagation host, only three rodent species contribute for more than 90% to the feeding of larvae, and for ~40% to the feeding of nymphs. Thrushes contribute for ~40% to the feeding of nymphs. These common rodent and bird species are not only the main contributors to the tick cycle, but also of the *B. afzelii* and *B. garinii* cycles, making the tick and pathogen cycles inseparable. Long-term trend analyses indicated that the length of the annual tick questing season has increased with one month annually since 1985. The surface area of tick-suitable habitats in The Netherlands has increased with ~20% over the last decades as well. Forest conversion from production forest to natural/recreational forest, not only increased the density of infected nymphs, but also the genetic diversity of the Lyme spirochete. Molecular epidemiological and experimental analyses revealed that rodents contribute most to the infection of ticks with *Borrelia afzelii*, *Neorhlichia mikurensis* and *Borrelia miyamotoi*. Birds contribute most to the infection of ticks with *Borrelia garinii* and *Rickettsia helvetica*. As a result, preferential co-infections occur in ticks, which in turn present co-exposure to, and possibly cause infections in humans. Indeed, we found *B. garinii* and *R. helvetica* co-infections in patients suspected for neuroborreliosis. The vertebrate hosts responsible for the maintenance of *B. burgdorferi* ss., *B. spielmanii*, and *B. bavariensis*, have not been identified yet. The development and future implementation of interventions in the tick or *Borrelia* cycle requires long-term and fruitful collaboration with nature reserve owners. For nature reserve owners, we developed and validated tools (by extensive fieldwork) for the generation of local risk-maps: “Hot spots” with high tick densities and visitors can be identified and a list of

relatively simple interventions (e.g. mowing) can be implemented. Field experiments have shown that fencing to exclude roe deer is a successful intervention to significantly reduce the density of ticks.

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## WG 1

# The “One Health” concept in the ecology of vector-borne diseases

## POSTER PRESENTATIONS

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## **COXIELLA BURNETII INFECTION AT THE IBERIAN IBEX-TICK INTERFACE IN NORTHEAST SPAIN**

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Q fever is a zoonotic disease caused by the bacterium *Coxiella burnetii* and ruminant livestock are its main reservoir. Nevertheless, little is known about the role of wildlife and ticks on Q fever maintenance and transmission. The Iberian ibex (*Capra pyrenaica*, Schinz 1838) is one of commonest ungulate species of the Iberian Peninsula. In the last decades, the Iberian ibex population of the National Game Reserve “Ports de Tortosa i Beseit” (PTiB, NE Spain), suffers from intense tick parasitization and low reproduction rate. This observation, potentially linked to *Coxiella* infection, has motivated the present study aimed at (1) detecting the presence of antibodies against *C. burnetii* in ibexes, *C. burnetii* DNA in ticks hosted by the same ibexes and assessing its relationship and (2) assessing the role of several factors in determining *C. burnetii* DNA presence within ticks. Serum samples and 673 ticks from 56 hunter-harvested ibexes were collected by Game Rangers in PTiB between 2012 and 2015. Detection of serum antibodies was performed with an ELISA test, while the presence of *C. burnetii* DNA in ticks was detected by PCR. The relationship between serum antibodies against *C. burnetii* in ibexes and *C. burnetii* DNA in ticks was evaluated by a contingency table. On the other hand, we used tree models to explore the effects of landscape structure on the likelihood of detecting *Coxiella* DNA in ticks. Eighteen percent of the ibexes (10/56; 95%CI, [10%-29.8%]) showed antibodies against *C. burnetii* and bacteria's DNA was detected in five of the six tick species, mainly in *Rhipicephalus bursa*. There was no relationship between the

serum antibodies of ibexes and *C. burnetii* DNA in the ticks carried by the same ibex. On the other hand, PCR positive ticks increased from 2014 onwards, particularly in areas close to small ruminant livestock and within oak forest ( $R^2= 0.70$ ).

## BIOINVASION OF VECTOR-BORNE FILARIOID NEMATODES IN THE ARCTIC AND BOREAL ECOSYSTEMS

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The nematode *Setaria tundra* appear to have emerged in Fenno-Scandinavian reindeer during the latter half of the 20<sup>th</sup> century, associated with peritonitis and the death of thousands of reindeer in 1973. A second outbreak in 1989 occurred among moose and caused peritonitis again. A third outbreak started in 2003 among reindeer leading to peritonitis and decreased welfare of reindeer and economic losses. At the same time, changes caused by *Onchocerca* spp., transmitted by blackflies (Simuliidae), were more and more common in reindeer and moose meat inspection. Infection caused chronic tarsitis and necrotic granulomas between muscles and scars in livers. In 2004-2006, new parasites were found in reindeer, inhabiting the ruminal lymphatic vessels, and were identified, for the first time in Europe, as *Rumenfilaria andersoni* Lankester and Snider, 1982 (Splendidofilariinae). This was, as far as we know, the first report of lymphatic-dwelling filarioids in ruminants anywhere. In Finnish reindeer, *R. andersoni* prevalence was locally up to 95 %. In moose, the observed prevalence was 10 %, in wild forest reindeer 69 %, white-tailed deer 15 % and roe deer 3 %. The impact of *R. andersoni* to cervid health is unknown but visible inflammatory changes were seen around lymphatic vessels. Recent data documents the geographical expansion of filarioid nematodes of ungulates to subarctic and that mean summer temperatures exceeding 14 °C drive the emergence of disease outbreaks due to *S. tundra*. Our current data, including genetic comparisons of North American and Finnish *R. andersoni* isolates, suggest that *R. andersoni* became established in Finland very recently, together with introduction of white-tailed deer from North America in 1935; subsequent invasion and emergence in the past 70-80 years perhaps has been driven by climate-related factors. As *R. andersoni* is found in all the four cervid species in Finland, it can be expected to spread further into naïve populations in

Eurasia. Mosquitoes transmit *S. tundra* and black flies *Onchocerca* spp., whereas the vector of *R. andersoni* is unknown. The development of *S. tundra* to the infective stage in mosquitoes is temperature dependent. We demonstrated that mean summer temperatures exceeding 14 °C drive the emergence of disease outbreaks due to *S. tundra*, but the morbidity manifests in the following summer, if again warm. This hypothesis was further supported in autumn 2014 following 2 consecutive exceptionally warm summers, leading to the emergence of the most recent outbreak of *S. tundra* and also *Onchocerca* in Finnish reindeer.

## MOSQUITO (DIPTERA: CULICIDAE) SPECIES DIVERSITY AND DISTRIBUTION IN TARTU, SOUTHERN ESTONIA

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Mosquitoes are widespread and numerous during the warm season in Estonia. However, mosquito species have not been studied in Estonia since 1954, leaving many unknowns about the local diversity of these insects. Since then, the nomenclature has changed and there is no data whether new mosquito species have emerged or previous ones withdrawn. The aim of this pilot study was to test our sampling methods; to investigate the diversity and space-time dynamics of mosquitoes in Tartu, a town of approximately 100,000 inhabitants. We hypothesized that the composition of the local mosquito population would change over the warm months as generally expected. This study was conducted in 2013, when 6 main and 3 additional sampling sites were selected around the downtown area of Tartu, which is halved by the river “Emajõgi”. Mosquitoes were collected from the sampling sites by performing 25 sweeps in 2 sets with a single insect net. In 2015, additional samples were collected by netting and with Mosquito Magnet® traps. Mosquitoes were mainly determined by morphological characteristics, but DNA sequencing was used to identify doubtful species. In total, 23 species were identified. The most numerous species were *Ochlerotatus communis* in 2013 and *Ochlerotatus punctor* in 2015. All the identified species are regarded as common for Estonia. The species diversity (by Shannon–Wiener index) did not significantly differ between sampling sites. However, the dominant species did fluctuate during the season. It seems that the netting protocol used in this preliminary study proved an adequate low cost method for surveillance of urban mosquitoes. This project will be continued by collecting and analysing mosquito species in other areas of Estonia, as well as testing their potential for distributing suspected infectious diseases.



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## PREVALENCE OF VECTOR-BORNE DISEASES IN STRAY DOGS FROM BELGRADE, SERBIA

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Climate change coupled with the following changes of the habitats, intensive pet travelling and animal importation, favor the expansion of the arthropod vectors as well as the canine vector-borne diseases (CVBDs) into formerly non-endemic regions. Some of CVBDs infectious agents have zoonotic potential and they are considered as a serious risk for human health. Stray dogs in urban areas can play an important role of CVBDs reservoirs, since they spend their whole life outdoors and are constantly exposed to the vectors, but without receiving any prophylactic measures against parasites. The aim of this study was to evaluate the prevalence of vector-borne diseases in stray dog population from Belgrade, capital of Serbia. EDTA blood samples from 86 clinically healthy animals were tested for the presence of *Dirofilaria immitis* antigen and antibodies against *Borrelia burgdorferi*, *Anaplasma* spp. and *Ehrlichia* spp. by using SNAP<sup>®</sup> 4Dx<sup>®</sup> Plus tests (IDEXX Laboratories, Inc., Westbrook, ME, USA). All blood samples were also examined for the presence of circulating microfilariae by modified Knott's test. Additionally, all dogs were subjected to an ocular examination in order to detect *Thelazia callipaeda* infection. A total of 23% (20/86) dogs were positive to at least one of the agents detectable by SNAP<sup>®</sup> 4Dx<sup>®</sup> Plus test. Infection with *D. immitis* was recorded in 12% (10/86) of animals and 8% (7/86) of dogs were positive for *Anaplasma* spp. No animals with mono infections caused by *Ehrlichia* spp. or *B. burgdorferi* were detected. Double infections (caused by *Ehrlichia* spp/*B. burgdorferi*, *Ehrlichia* spp/*Anaplasma* spp. and *D. immitis*/*B. burgdorferi*) were recorded in 3% (3/86) of dogs. Microfilariae were detected by modified Knott's technique in all *D. immitis* antigen-positive dogs. In addition, 20% (17/86) of animals were infected only by *T. callipaeda* and 7% (6/86) of *Thelazia*-positive dogs were co-infected with some of the SNAP detectable CVBDs. Considering the observed prevalence and zoonotic character of almost all identified CVBDs, A comprehensive stray dog management approach is needed in order to protect health and welfare of stray dogs, client-owned animals and human population.

## EXPERIENCES IN DEVELOPING COLLABORATIVE TOOLS FOR TEACHING VETERINARY PUBLIC HEALTH: A MULTILINGUAL ATLAS/MANUAL ON PARASITOLOGY AND PARASITIC DISEASES & ZOONOSES, BY THE SAPUVENET III PROJECT

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SAPUVETNET III “Contributing to the Millenium Development Goals through the One Health Approach”, is a project co-financed under the EU ALFA program (n. DCI-LA/2008/75) aimed to support an International network on Veterinary Public Health (VPH) constituted by Faculties of Veterinary Medicine from Latin-America (LA) and Europe (EU) (<http://www.sapuvetnet.org>). Since its start, SAPUVETNET has envisaged a series of objectives/activities aimed to promote and enhance VPH research/training and intersectoral collaboration across LA and EU. Didactic tools developed by SAPUVETNET can be used for distance learning can be freely circulated/distributed, and modified/adapted to the local context of any country/geographical area, even outside LA and EU. Amongst the various didactic/teaching material produced by the Project, a multilingual *Atlas/Manual on veterinary parasitology and parasitic zoonoses* was developed by some SAPUVETNET partners. This product originates from a Dissertation work in Veterinary Medicine, entitled “(Veterinary) Parasitology from A to Z” (in Italian), and it is now available online (Beta version). The *Atlas/Manual* illustrates the variety, even if not complete, of parasites/diseases which may affect pets, livestock, and humans (zoonoses). We have also included a section on parasitic diseases of South American camelids which are very important in different member countries of SAPUVETNET. We have tried to give a great importance to the graphics (photographs, drawings, figures, etc.) to facilitate recognition of parasites and diseases that they cause. The

*Atlas/Manual* also includes a final self-assessment test (multiple choice questions) divided into two parts: a practical part is based on the recognition of parasites (photo of adults, intermediate phases and forms of resistance) and a theoretical part consisting of a series of questions to assess the level of knowledge of general concepts. The *Atlas/Manual* is to be considered an ongoing collaborative project, and the Authors/Editors are grateful to all colleagues willing to contribute for the improvement and completion of this didactic tool (e.g. revising the text, providing photos and/or drawing of parasites not yet available, and translating/adapting the *Atlas/Manual* to additional languages).

## BIO-GUIDED FRACTIONATION AND IDENTIFICATION OF LEISHMANICIDAL COMPOUNDS FROM ESSENTIAL OIL OF CHAMOMILE, *MATRICARIA RECUTITA* L

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Leishmaniasis is among the most problematic protozoan diseases causing a considerable morbidity throughout the world. It is an endemic disease that has been estimated at 300,000 new cases each year, most of them in children. The treatment of these infectious diseases including *Leishmania amazonensis* remains a challenging public health need. So, it is important to the search of promising approach to identify new natural, safe and effective alternative to treat this disease. In this respect, Chamomile (*Matricaria recutita* L), it is an annual herbaceous plant of the Asteraceae family, known by its therapeutic and medicinal virtues and has several biological activities such as antimicrobial, antioxidant, anti-inflammatory, and gastrointestinal protective. The purpose of the present study is to evaluate *in vitro* antiparasitic activity of extracts and essential oil from flowers of chamomile against *L. amazonensis* and bi-guided fractionation bioassays to isolate and identify molecules responsible for studied activity. Initially, chamomile essential oil and different extracts obtained using solvents of increasing polarity (water, methanol, chloroform and hexane) were evaluated against promastigotes of *L. amazonensis*. This activity was determined by the modified Alamar Blue®. Essential oil fractionation was guided by its inhibitory activity. Initially, it was injected over a silica gel column. Fractions obtained were combined in 6 fractions according to their TLC profiles. Based on the observed antiprotozoal activity, the



most active fractions were chromatographed on syphadex LH-20 and silica gel column. Recuperated fractions were then submitted to centrifugal preparative TLC using a chromatotron. Most active fractions were finally analysed by NMR. In the present study, it was observed that the essential oil exhibits higher *in vitro* anti-leishmaniasis activity than chamomile extracts. This activity was expressed by a low inhibitory concentration at 50% ( $IC_{50}$ ) ranging between  $(10.936 \pm 3.637 \mu\text{g/ml})$  for the essential oil to  $(15.977 \pm 0.246 \mu\text{g/ml})$  for the most active isolated fraction. NMR of this fraction analysis a complex mixture of molecules identified as sesquiterpenic compounds. In conclusion, these *in vitro* data suggest that the therapeutic use of sesquiterpens can be considered a promising alternative in the treatment of leishmaniasis caused by *L. amazonensis* species.

## IMPORTED MALARIA IN BULGARIA IN THE XXI CENTURY

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Malaria has been endemic in Bulgaria since ancient times. After the eradication of the disease in 1965, the country entered a phase of prevention of the reintroduction. In 1995-1996, a vivax malaria outbreak with 18 autochthonous cases was registered in Blagoevgrad region in South-Western Bulgaria. The sources of infection were migrants who stayed several weeks in the region before trying to cross illegally the border with Greece. Malaria is a mandatory notifiable disease in Bulgaria and a questionnaire is filled for each case. All cases are reported to the National Centre of Infectious and Parasitic Diseases where samples are sent for diagnosis confirmation and species identification by light microscopic examination of Giemsa-stained thick and thin blood smears. Between 2001 and 2014, 135 laboratory confirmed cases of imported malaria were registered in the National Centre of Infectious and Parasitic Diseases. Majority of them (n=98) were Bulgarian citizens who acquired the infection in Africa (95.9%), Asia (3.1%) and South America (1%). The rest (n=37) were foreign citizens of whom more than a third (n=14) were migrants from Afghanistan infected predominantly (85.7%) with *Plasmodium vivax*. Of all imported cases, 60.7% were registered during the anopheline activity season (April – October). Historical and recent records show presence of 13 anopheline species in Bulgaria: *Anopheles algeriensis*, *A. atroparvus*, *A. claviger*, *A. daciae*, *A. hyrcanus*, *A. maculipennis*, *A. marteri*, *A. melanoon*, *A. messeae*, *A. plumbeus*, *A. pseudopictus*, *A. sacharovi* and *A. superpictus*. Of them, *A. maculipennis*, *A. sacharovi* and *A. superpictus* were proven vectors in the past. Hand collection of indoor-resting mosquitoes performed inside animal shelters in three different regions showed that *A. maculipennis* comprises more than 95% of the mosquito population in the same area where malaria outbreak was recorded in 1995-1996. *A. sacharovi* accounts for 35% of the anopheline population in the Black Sea coastal area. Under the existing favourable climatic and faunistic conditions, migrants from malaria-endemic countries can become a source of infection for native anopheline

mosquitoes, with subsequent transmission to the local population and emergence of new autochthonous cases of malaria. This is particularly valid for patients infected with Asian strains of *P. vivax*, as proven by the local outbreak in 1995-1996 and the epidemic in Greece in 2011.

## RESERVOIR COMPETENCE OF INTRODUCED RACCOONS FOR HEMOPARASITES IN WESTERN POLAND

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Raccoon (*Procyon lotor*) is an opportunistic carnivore, native to North America which has been introduced to Japan and Europe, including Poland. Little is known about the potential ecological impact of raccoon introduction into Europe, although it has been suggested that high densities of raccoon could increase the predation pressure on native prey and the possible transmission of pathogens to humans as well as wildlife, domestic and captive animal populations. Do date, haemoparasites have not been recorded from raccoons in Europe. Therefore the purpose of this study was to investigate the presence of haemoparasites in polish population of raccoons and determine if this invasive species serves as reservoir host of potentially zoonotic pathogens. Both blood and spleen samples were obtained from raccoons (n=78) in Western Poland. The choice of genetic markers and primers was based on the literature data, mainly from research reporting pathogens of introduced raccoons in Japan, and our preliminary results, i.e. *B. burgdorferi* s.l. - *flaB*, 16S and 23S; Anaplasmatatacae - 16S, *groEL* and *gltA*; *Babesia* spp.- 18S; *Rickettsia* spp. – *gltA*. PCR methods were used for the detection of DNA of examined pathogens. All samples were simultaneously tested with the use of all genetic markers. Selected PCR positive products were purified and sequenced. BLAST searches were conducted in order to elucidate any homologies with previously deposited sequences in GenBank. Results of our preliminary study indicate that raccoons introduced to Poland could be reservoirs of Anaplasmatatacae pathogens, including *A. phagocytophilum*. Analyses showed that obtained *A. phagocytophilum* belongs to European zoonotic ecotype I proposed by Jahfari et al. (2014). Our investigation demonstrated a poor reservoir competence of raccoons for other examined species of haemopathogens. Thus, this current low incidence of infection could be te result of a relatively still new vector-pathogen-host relationships attributable to raccoons recently naturalized in Poland.

## WG 2

# Barcoding, molecular diagnosis and next generation sequencing

## ORAL PRESENTATIONS

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## MICROSATELLITE VARIATION AMONG DIVERGING POPULATIONS OF *DERMACENTOR RETICULATUS*

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*Dermacentor reticulatus* is the second most reported tick species after *Ixodes ricinus* in Europe. Geographic distribution of these ticks is fragmented. Five microsatellite loci were assayed for 23 populations (Latvia, Lithuania, Eastern and Western Poland, Germany, Czech Republic, Slovakia, Croatia, Spain and Ukraine) from three regions (Western-, Central- and North-Eastern Europe) spanning the range of distribution, and the levels and distribution of genetic variation were estimated. A total 232 ticks were investigated. Results of analysis showed that most of the molecular variability is contained within populations, and that populations cluster together similarly to known geographic distribution. Genetic drift and limited gene flow was considered to be important in determining the genetic structure within regions. Genetic differentiation  $F_{ST}$  values ranging from 0.014 to 0.256. Neighbour-joining dendrograms, Bayesian-clustering and PCoA analyses showed two genetic groups of *D. reticulatus* ticks: Western Europe and Eastern Europe. Eastern Europe group showed no evidence of clusterization according to geographical distribution patterns with low molecular variability between populations. Low genetic differences between Lithuanian, Latvian, Eastern Poland and Slovak populations of *D. reticulatus* show close relatedness among these populations.

## MOLECULAR PHYLOGENY OF IXODIDAE BASED ON 5 MITOCHONDRIAL AND NUCLEAR GENES WITH A FOCUS ON THE IXODES GENUS

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Ticks are the most important vectors of disease-causing pathogens in domestic and wild animals and are only second after mosquitoes as vectors of human diseases. With more than 900 species described to date, ticks (Ixodida) are a species-rich group. With at least 3 families and 18 genera recognized, their biodiversity results from several hundred million years of evolution. Although morphological characters may provide useful information to recognize common ancestors and establish relationships among species, the identification of sub-genera (for example in the species-rich *Ixodes* genus, with 250 species described) has never been accepted by a consensus of tick taxonomists. Our knowledge of the phylogenetic relationships among ticks, mostly based on non-coding (ribosomal) genes, remains largely incomplete. To provide a better understanding of tick evolution and systematic, we have conducted a molecular phylogeny of 27 species of ticks (including some species for which no sequences have been deposited to date in GenBank to our knowledge), with a special focus on the *Ixodes* genus (21 species, including representative species of several sub-genera recognized by several tick taxonomists). Species from different biogeographic regions have also been selected for the calibration of a molecular clock within the Ixodida. Five genes have been sequenced, including 2 ribosomal genes (1 being also mitochondrial) and 3 coding genes – the

reading frame allowing more easily the recognition of insertion-deletion events hence facilitating the alignment - (1 being also mitochondrial) providing an alignment of more than 5000 nucleotides on which several phylogenetical reconstruction methods have been applied. The monophyly of the Amblyommidae is confirmed by our phylogenetic analysis. However, several morphologically-recognized *Ixodes* sub-genera were not forming monophyletic groups. The consequences of those finding on tick systematic, on our understanding of tick evolution and on applications for vector-borne control will be discussed.

## MOLECULAR DETECTION OF *THEILERIA*, *BABESIA*, AND *HEPATOZOON* SPP. IN IXODID TICKS FROM PALESTINE

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Ixodid ticks transmit various infectious agents that cause disease in humans and livestock worldwide. A cross-sectional survey on the presence of protozoan pathogens in ticks was carried out to assess the impact of tick-borne protozoa on domestic animals in Palestine. Ticks were collected from herds with sheep, goats and dogs in different geographic districts and their species were determined using morphological keys. The presence of piroplasms and *Hepatozoon* spp. was determined by PCR amplification of a 460–540 bp fragment of the 18S rRNA gene followed by RFLP or DNA sequencing. A PCR-RFLP method based on the 18S rRNA was used in order to detect and to identify *Hepatozoon*, *Babesia* and *Theileria* spp. A total of 516 ticks were collected from animals in six Palestinian localities. Five tick species were found: *Rhipicephalus sanguineus* sensu lato, *R. turanicus*, *R. bursa*, *Haemaphysalis parva* and *H. adleri*. PCR-based analyses of the ticks revealed *Theileria ovis* (5.4%), *Hepatozoon canis* (4.3%), *Babesia ovis* (0.6%), and *Babesia vogeli* (0.4%). *Theileria ovis* was significantly associated with ticks from sheep and with *R. turanicus* ticks ( $p < 0.01$ ). *H. canis* was detected only in *R. sanguineus* s.l. and was significantly associated with ticks from dogs ( $p < 0.01$ ). To our knowledge, this is the first report describing the presence of these pathogens in ticks collected from Palestine. Communicating these findings with health and veterinary professionals will increase their awareness, and contribute to improved diagnosis and treatment of tick-borne diseases.

## RE-CREATING THE DISTRIBUTION OF *IXODES RICINUS* IN FINLAND

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The distribution of the tick *Ixodes ricinus* in Finland was published 1961. Spatiotemporal data on cattle and bovine babesiosis caused by *Babesia divergens* was compiled from official statistics (1883-2015) and 55 historical newspapers (1750-1910). This data was tested with those of other cattle diseases. Since ticks are vectors of *B. divergens* and the cattle grazed in forests, it is possible to reconstruct the minimum historical distribution of ticks in Finland. Cattle were until 1960 the only significant large ruminant in the Finnish forests. The historical data was also compared with available corresponding data from Sweden and Norway. The distribution of bovine babesiosis 1850-1910 and 1950-1959 shows a stable northern limit from approximately the end of the river Tornio by the Swedish border southeast to Kuhmo by the Russian border. It coincides with the known distribution of *I. ricinus* in 1952-1959 and 2014 and also with some dung beetles. It also correlates with the northern limit of human villages in Finland in 1560. Beyond this line finds of *I. ricinus* can be explained by ticks on dogs and other pets brought there by tourists. Ultimately the line can be explained as a general soil fertility border. Northeast of this border it has been difficult for the villages to get fodder for the animals and crops from their fields. For *I. ricinus* it is difficult to find an enough thick layer of detritus for hibernation. The number of cases of babesiosis and the tick population were at their highest in 1960, when there were 2.2 million cows. Then the agricultural policy changed. Forest grazing and babesiosis declined rapidly. Cattle population dropped to one million kept in enclosed pastures. Ticks lost their main blood resource and declined. The populations of Eurasian elk, then also white-tailed deer and roe deer started to increase after 1970. The presently growing tick population is a result of that increase. Today there are after hunting season approximately 100,000 elks, 40.000 white-tailed deer and 20.000 roe deer. The number of ruminants in the forests is now about 10 % of the cattle in 1960, so with an increasing ruminant population the density of the tick population in Finland is likely to grow still.

## MICROMAMMALS AS RESERVOIRS OF EMERGING ZONOTIC PATHOGENS IN ROMANIA

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Micromammals play an important role in the life cycle of ticks and are reservoir hosts for several emerging zoonotic pathogens. The aim of this study was to investigate the occurrence of *B. burgdorferi* s.l. in rodent tissue samples and in engorged *I. ricinus* ticks in Romania. During 2010 – 2011, eleven micromammal species were trapped using conventional methods. Tissue samples, heart and liver, were analyzed by qPCR targeting the *ospA* gene of *B. burgdorferi* s.l. Positive samples were further analyzed by conventional PCR and sequencing. In total of 395 heart and 399 liver samples from 401 animals were individually analyzed. The overall prevalence of infection with *B. burgdorferi* s.l. in tissue samples was 4.5%. The most commonly detected spirochete species were *B. afzelii* (80% from the total infected) followed by *B. garinii*, respectively *B. burgdorferi* s.s. (10%). *Borrelia afzelii* was detected in *Apodemus agrarius*, *A. flavicollis*, *Crocidura leucodon*, *C. suaveolens*, *Micromys. minutus*, *Microtus agrestis*, *M. arvalis* and *Myodes glareolus*. The following host-pathogen associations were registered: *B. garinii* - *A. agrarius*/*M. arvalis*, respectively *B. burgdorferi* s.s. - *A. agrarius*/*M. arvalis*. Our study reports for the first time the occurrence of *B. burgdorferi* s.l. in the micromammals from Romania. The presence of different spirochetes species in the micromammalian tissues reveals the importance of animal surveillance regarding Lyme borreliosis, thus, possibly these animals can pose a public health risk.

## VIROME ANALYSIS OF TICKS

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The global incidence of tick-borne disease is increasing worldwide due to environmental changes. Therefore, the risk of emergence of tick-borne diseases is now considered as significant. Although ticks have the potential to transmit many different viruses, most studies surveying tick-borne pathogens in Europe have focused on bacterial and/or parasitic pathogens. Numerous reports detailing parasitic or bacterial prevalence in either European ticks or animal reservoirs are published every year. For viruses however, the situation is completely different. Only few data are available regarding prevalence of tick-borne viruses (TBVs) in Europe, mainly Tick-borne encephalitis (TBE) virus and Crimean-Congo hemorrhagic fever (CCHF) virus. Several TBVs have already emerged in specific geographical regions, such as TBE, Powassan and CCHF, while novel TBVs are continually being discovered (severe fever with thrombocytopenia syndrome virus (SFTSV), Bourbon virus, etc.). These factors highlight the importance of increasing the knowledge about tick virome. To analyze the virome of *Ixodes ricinus* ticks, the main vector of tick-borne pathogens in Europe, we used two complementary approaches: high-throughput sequencing (HTS) to detect all viruses present in ticks (expected, unexpected and new) and high-throughput real time PCRs for prevalence studies. With HTS approaches, we identified 545 assembled contigs related to eukaryotic viruses. The vast majority of hits mapped to families known to include arboviruses, and the greatest number of contigs likely designated a novel *Nairovirus* and a new *Phlebovirus*. The only known virus identified in the ticks was the Eyach virus, first isolated in Europe in the 80s, and which has not since been studied anymore. We then demonstrated its capacity to



multiply and persist in the blood of OF1 mice, and its neurotropism in mice. With high-throughput real time PCRs, a large scale epidemiological study was conducted on 20,163 *I. ricinus* nymphs collected from five European countries. This advanced methodology permitted the simultaneous detection of 22 tick viral species (from different viral families) across 47 pool of ticks. We successfully determined the prevalence of expected (TBE, Uukuniemi), unexpected (Nairo-like) and rare (Eyach) viruses in the five European countries. Some of them were detected for the first time in France and in the Netherlands (Eyach and Nairo-like). These complementary new high-throughput methods allowed us to detect and characterize a high number of TBVs, particularly new ones, present in ticks, mammals or humans. They also represent a major improvement in epidemiological studies, which greatly facilitates prevalence studies of TBVs in ticks.

## TICKS AND TICK-BORNE DISEASES IN SHEEP ON THE ISLAND OF LESBOS/GREECE. A GIS BASED APPROACH

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Epidemiological data on tick borne diseases affecting small ruminants in Greece remain scarce. The present study aimed at: 1) assessing tick species presence and distribution on the sheep population of Lesbos Island and 2) screening both sheep blood and tick samples for the presence of 45 pathogens belonging to the genera *Anaplasma*, *Ehrlichia*, *Babesia* and *Theileria* by the application of PCR/RLB. In total 101 farms were visited using a GIS based grid approach followed by proportional allocation (the number of farms visited in each quadrant was proportional to the total number of farms in each quadrant) during May, June, September and October 2015. During the study 505 blood samples (5 animals/farm) and 1706 ticks from the respective number of sheep were collected. In 72.3% of the farms tick infestation with 9 different species throughout the collection period was observed. During the May-June sampling period *Rhipicephalus turanicus* was the predominant species found, accounting for 95.2% (May) and 84.6% (June) of the collected ticks during the respective month. *R. sanguineus*, *R. bursa*, *Hyalomma marginatum* and *H. excavatum* were also collected in small numbers. During autumn *Dermacentor marginatus* (48.8% of collected ticks) and *Haemaphysalis parva* (43.7%) predominated when both September and October samplings were considered. In addition *H. sulcata*, *H. punctata* and *R. bursa* adults were collected in small numbers. Blood PCR/RLB analysis from the May-June collected samples (39 farms/195 samples) revealed that 97.4% of the animals were single (*Anaplasma ovis*, *Theileria ovis*,

unknown A/E and unknown B/T) or mixed (*A. ovis*+*T. ovis*, unknown A/E+*T. ovis*, unknown B/T+*A. ovis*, *Babesia ovis*+*A. ovis*, unknown A/E+*B. ovis*, unknown A/E+*B. rossi*) infected with six different pathogens. 59.3% and 64.9% of the animals were infected with *A. ovis* and *T. ovis* respectively, either alone or in combination with other pathogens. Infections with *B. ovis* accounted for 1.0% of the screened blood samples, whereas a considerable number of animals were infected with unknown A/E and B/T species. Analysis of the remaining blood samples and a representative number of the predominant tick species (*R. turanicus*, *D. marginatus* and *H. parva*) is still ongoing and results will be presented during the meeting. The above data provide a first comprehensive insight into the tick fauna and tick borne pathogens infesting/infecting the sheep population of the island. Analysis by PCR/RLB will provide interesting data about the role of the three predominant species (*R. turanicus*, *D. marginatus* and *H. parva*) as vectors of tick borne pathogens.

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## EMERGING HORIZONS FOR TICK-BORNE PATHOGENS: FROM THE "ONE PATHOGEN-ONE DISEASE" VISION TO THE PATHOBIOME PARADIGM

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Ticks as vectors of several notorious zoonotic pathogens, represent an important and increasing threat for human and animal health in Europe. Although the most frequent tick-borne disease reported in European citizens is Lyme Borreliosis with more than 65 000 new cases each year, patients bitten by ticks can also be exposed to many other micro-organisms. These include viruses, bacteria and parasites, some of which were identified in ticks decades prior their association with human disease, whereas others were discovered only recently and their public health importance remains so far unknown. Recently, the use of next-generation sequencing (NGS) technology revealed that ticks harbour, in addition to pathogens, many other micro-organisms which co-exist and might interact with pathogens. Our vision of tick-borne pathogens therefore has evolved to a more integrated view, which considers the "pathobiome", representing the pathogenic agent integrated in its biotic environment including other pathogens, commensals and symbionts. In this presentation we

will describe the tick pathobiome by using different combined high through put methods (without a priori high throughput sequencing and high throughput qPCR) and we will analyse how this new vision will change our understanding of tick-borne diseases and will discuss the implications in terms of research to efficiently prevent and control the threat posed by ticks in Europe.

## MOLECULAR EPIDEMIOLOGY OF HUMAN CUTANEOUS LEISHMANIASIS IN JERICHO AND ITS VICINITY FROM 1994 TO 2015

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Cutaneous leishmaniasis (CL) are vector-borne parasitic diseases endemic in many countries of the Middle East including Palestine. Between 1994 and 2015, 2160 clinically suspected human cases of CL from the Jericho District were examined. Stained skin tissue smears and aspirates were checked by microscopy and cultured for promastigotes, respectively. For leishmanial species identification, amplification products from a PCR-ITS1 followed by RFLP analysis using Hae III. Data were analyzed using EpiInfo free-software. The overall infection rate was 41.4% (895/2160), 56.3% (504/895) of the cases were male, 43.7% (391/895) female, 60.5% (514/849) children under age 14, 41.3% (259/627) of the cases were caused by *L. major* and 57.3% (359/627) by *L. tropica*. The case numbers peaked in 1995, 2001, 2004, and 2012. Statistically-significant clusters of cases caused by *L. major* were restricted to the Jericho District; those caused by *L. tropica* were from the Districts of Jericho, Bethlehem, Nablus and Tubas. CL is seasonal and trails the sand fly season. Distribution of cases was parabolic with fewest in July. The monthly total number of cases of CL and just those caused by *L. major* correlated significantly with temperature, rainfall, relative humidity, evaporation, wind speed and sunshine ( $P < 0.05$ ,  $r^2 = 0.7-0.9$  and  $P < 0.05$ ,  $r^2 = 0.5-0.8$ , respectively). Cases caused by *L. tropica*, significantly, had a single lesion compared to cases caused by *L. major* ( $P = 0.0001$ ), which, significantly, had multiple lesions ( $P = 0.0001$ ). This and previous studies

showed that CL is present in all Palestinian districts. The surveillance of CL has increased public awareness and molecular biological methodology for leishmanial species identification is an essential addition to classical diagnosis. The overall results are discussed, correlated to climatic and environmental changes and large-scale human activities.



## SAND FLIES AND VIRUSES: 10 YEARS OF INTEGRATED RESEARCH LEADING FROM FIELD STUDIES TO THE CLINICAL STUDIES

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Several phleboviruses (Bunyaviridae), transmitted by phlebotomine sand flies belonging to the genus *Phlebotomus* in the Old World, can infect humans and cause mild to severe diseases. The last decade has witnessed the discovery of unprecedented quantity of new phleboviruses the pathogenicity of which is poorly understood. There is no commercially available molecular test for the detection of these viruses, even for the viruses which have been demonstrated to be of medical interest. Therefore our aim was to propose for researchers and diagnostic laboratories an arsenal that may be used in any laboratory with real-time PCR equipment. Next generation sequencing has allowed to increase drastically the number of complete sequences for these viruses. These sequences have been used to verify the ability of published molecular assays to be used in diagnostics settings as recently done for Chikungunya and Zika viruses (<http://www.european-virus-archive.com/article147.html>; Charrel et al 2016 Bull WHO). Attempts to answer this question necessitate the availability of diagnostic tools that must be specific and sensitive. We will review the current situation and will present the tools that have been developed in the framework of European collaborative projects in order to publicize their use for field studies and clinical studies with specific interest for phleboviruses with demonstrated medical importance. The objectives were (i) to compile and align all complete sequences for each of the 5 viral genes, (ii) to match primers and probes published in the literature to define in silico their capability to be used for discovery or diagnostic studies, (iii) to design diagnostic assays with improved performances, (iv) to test these newly developed systems against a panel of viruses representative of the genetic diversity, (v) to prepare the selected assays in a freeze-dried format and make them accessible for researchers and clinical medical and veterinary microbiologists through the European Virus Archive [EVA] (<http://global.european-virus-archive.com/>) platform together

with suitable inactivated material to serve as quantitative positive controls. As a result of this study, the 4 first objectives have been fulfilled, and the last objective is being undertaken to provide preliminary results during the COST conference: (i) to compile and align all complete sequences for each of the 5 viral genes: sequences were either retrieved from the Genbank database or provided by research groups involved in this study. (ii) to match primers and probes published in the literature to define: data mining has been performed to compile all articles in which primer- and probe-sequences were disclosed; matching with aligned genomic sequences was done manually. (iii) to design diagnostic assays with improved performances: improvement/degeneration of published primers were done manually; conserved patterns (universal for all phleboviruses / universal for sandfly-borne phleboviruses belonging to the 3 species present in the Old World / Species-specific primers were identified visually and used to design candidate diagnostic assays. (iv) to test these newly developed systems against a panel of viruses: virus strains were obtained from the EVA collection. (v) to prepare the selected assays in a freeze-dried format: the ready-to-use reagents were prepared according to a standardized procedure that is routinely used for preparing diagnostic reagents employed in the largest French clinical virology laboratory for medical diagnosis. In conclusion, we have developed the capacity for diagnosis of sandfly-borne phleboviruses that is now easily accessible and may help to better understand the medical impact of these viruses in human and veterinary medicine.

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## A SUBGROUP OF SWEDISH *Aedes vexans* IS REPRODUCTIVELY ISOLATED, AS DETERMINED BY MITOCHONDRIAL AND NUCLEAR MARKERS

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The flood water mosquitoes *Aedes vexans* and *Aedes sticticus* are a nuisance problem in several areas of Sweden and are predicted to spread and become more common due to changes in precipitation and temperature as an effect of global warming. In addition to the nuisance problem *Aedes vexans* also have the potential to act as a vector for more than 30 viruses and many important disease viruses such as West Nile virus, St. Louis encephalitis and eastern equine encephalitis. In the event of an outbreak of a vector borne disease, previous knowledge of distribution and population dynamics of the vector can help to focus resources to the right locations to prevent spread of the disease. In addition to knowledge about presence of a vector such as *Aedes vexans* in different locations it may be important to also have knowledge about the intraspecific genetic variation and intermixing of mosquito populations from different locations. In our study we have analyzed 80 mosquitoes morphologically determined as *Aedes vexans* collected at eight locations throughout Sweden in order to assess genetic differences. For the COI marker in the mitochondrial DNA a subset of 22 of the analyzed mosquitoes cluster together apart from the other samples with an intergroup distance of 12.9%. We further sequenced two nuclear loci, ITS2 and the homolog of *Aedes aegypti* gene AAEL000094 from the nuclear DNA and find that the same mosquitoes also group apart from the others using these markers. These results indicate that this group represents a reproductively isolated population among morphologically determined *Aedes vexans*. 57 of 80 specimens have been collected in two areas where both groups, according to COI sequence, are present. Comparison with COI sequences from previously sequenced European *Aedes vexans* show that two specimens collected in Belgium group together with the Swedish outgroup suggesting that this genotype is present throughout northern Europe. Our results thus suggest that there is a cryptic taxonomic unit related to *Aedes vexans* in northern

Europe. It will be of utmost interest to see whether this variant differs in vector competence or life cycle characteristics.

## TOWARDS HIGH-THROUGHPUT IDENTIFICATION OF ARTHROPOD VECTORS BY MASS SPECTROMETRY

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Accurate and high-throughput identification of vector arthropods is of paramount importance in surveillance and control programmes which are becoming more common due to changes in the geographic range and extent of many arthropod-borne diseases, also in Europe. Protein profiling by matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) technically fulfils these requirements for identification, and reference databases have recently been established for some vector taxa by various research groups. However, their approaches greatly vary in terms of sample processing, equipment used, data acquisition and analysis. Furthermore, these databases have typically been generated on a project-by-project base, are stored on local drives ('in-house databases') and thus are not accessible to the public. We have established the largest database with regard to vector arthropods, including Ceratopogonidae, Culicidae, Ixodidae, Phlebotominae and Simuliidae, and we continually expand it in collaborative work, with the goal of creating a comprehensive, centralized database that comprises the reference spectra of all major arthropod vectors. The database is maintained by a private company (Mabritec SA) as this ensures both a customer-oriented service and a greater sustainability. Further, we for the first time showed that spectra obtained on mass spectrometers from different companies can be analysed using this database. In order to render the MALDI-TOF MS technology universally useful and available for the identification of arthropod vectors, we have developed harmonised protocols ('standard operating procedures' [SOPs] including written and illustrated material as well as short film sequences) to provide instructions on arthropod handling and processing as well as on data acquisition on different instruments. Thus, anyone with access to a MALDI-TOF MS machine can create mass spectra of arthropod vectors on this equipment and obtain species identification by electronically submitting the data to Mabritec SA for comparison with the

established centralised database. The ultimate aim is an on-line database with access to the arthropod module for trained users. Ideally, a public core-funding would assure free access for non-commercial institutions and maintenance of the database (with regard e.g. to continued expansion, taxonomic adaptations).

## BITING MIDGES: VECTORS OF WILD RUMINANT PATHOGENS?

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Biting midges of the genus *Culicoides* play a crucial role in the transmissions of various pathogenic organisms. Most notably, they are vectors of several arboviruses which might have a serious impact on livestock (e.g., Blue tongue disease); however their impact on wild ruminants and the involvement of culicoids in parasite transmissions within wildlife are widely unknown. In our study, we focused on detection of two groups of parasites, trypanosomatids and filaria, in biting midges. In addition to the vector surveillance, we have screened occurrence of the interest parasite groups in blood and skin samples of several hundred (semi)wild ruminant hoofed games collected in several localities in the Czech Republic to solve the potential involvement of biting midges into the parasite transmissions. Using a specific PCR detection and SSU sequencing, two species of monoxenous trypanosomatids, *Herpetomonas pessoai* and one undescribed species of the „jaculum“ clade, were identified in non blood-fed biting midges caught in the proximity of wild ruminants. Apart from these two trypanosomatids, both found in culicoids for the first time, two undetermined filaria species were detected by PCR and COI sequencing. In blood and skin samples from wild ruminant, five genotypes of *Trypanosoma theileri/cervi* complex and four genera of filaria worms, *Setaria*, *Onchocerca*, *Cercopithifilaria* and *Mansonella*, were detected. Very high host specificity and partially also tissue specificity were detected for two filaria species: *Cercopithifilaria rugosicaudata* parasitize exclusively fallow deer was found mostly in their neck skin and *Onchocerca flexuosa* predominantly found in red deer with localisation mostly in their abdominal parts. The main goal of the study was to detect whether the biting midges and their ruminant hosts, hoofed games, share the same parasites and whether the

biting midges could play a role as vectors. However absence of dixerous genus *Trypanosoma* and the filaria species detected in hoofed games in culicoids collected in the proximity of wild ruminants indicate that biting midges are probably not involved in the transmission cycles of these parasites.



## EXPLORING BALKAN SAND FLY FAUNA USING MALDI-TOF MASS SPECTROMETRY

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Bloodsucking females of phlebotomine sand-flies are the only proven vectors of *Leishmania* parasites, causative agents of leishmaniasis. Their conclusive species identification in endemic areas, where morphologically similar species with different vectorial capacity and ecology occur, is profound. Traditional morphological approach relies on minute and sometime dubious characters; therefore several molecular methods were applied recently. Protein profiling by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry is a promising technique for identification of various organisms including insects. It is simple, accurate, and requires minimal sample preparation. The obtained mass spectrum represents a characteristic and unique protein fingerprint, which allows rapid and effective species identification. We optimized methods of specimen capture, storage, sample preparation, tested suitability of different body parts of sand fly for protein identification, and developed a multi-approach protocol that utilizes a single insect body for several identification methods (morphological, DNA and protein based). Further on, the influence of blood meal at several time intervals after feeding on the quality of protein profile of females was investigated. Also spectra of larvae (L4) and pupae were measured and compared with profiles of adult sand flies. A reference spectra database comprising 16 sand fly species was established using MALDI Biotyper software. It includes species maintained in laboratory

colonies as well as field-collected specimens identified by morphological analysis and DNA sequencing. During two field trips to Balkan countries (Bosnia and Hercegovina, Bulgaria, Croatia, Hungary, Macedonia, Montenegro, Serbia and Slovenia) in summer 2015, 680 specimens (*Larroussius*, *Adlerius*, *Paraphlebotomus*, *Phlebotomus*, *Sergentomyia*) out of 7257 totally captured were identified by protein profiling which proved as reliable. It was useful for female specimens of the subgenus *Larroussius* (492 analyzed specimens) and differentiation between its closely related species (*P. neglectus*, *P. perfliewi*, *P. tobbi*). Also two species of the subgenus *Adlerius* (*P. balcanicus*, *P. simici*), hardly distinguishable by morphology, were unambiguously differentiated by protein spectra. All dubious samples (difference between morphological and protein based ID) were clarified by a sequencing analysis, which confirmed the identification according to MALDI-TOF MS in all cases. In summary, MALDI-TOF protein profiling is a suitable, time- and cost- effective method for species identification of large sets of field-caught sand flies if these are collected, stored and analyzed using a standard, optimized protocol.

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## VECTORS OF EQUINE INFECTIOUS ANAEMIA: AN ASSESSMENT OF THEIR PRESENCE AND DISPERSION POTENTIAL IN BELGIUM

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The recent outbreaks of equine infectious anaemia (EIA) in Belgium have dramatically increased the concern about this viral disease and its transmission in our country. Mechanical transmission seems to be the major route of spread of EIA with Tabanidae and Muscidae considered as the main arthropod vectors. Therefore, this context requires a reinforcement of surveillance about potentially emerging diseases and point to a critical need of updated data on its arthropod vectors but also on their potential transmission efficiency and the potential expansion of this virus applied to the Belgian context. H traps were placed in pastures with horses in the vicinity and located in different ecological environments across Belgium during 2 successive seasons (2014 and 2015). Additionally, sticky black boards were fixed in several places inside horse riding establishments across Belgium. Morphological identification was implemented on Tabanidae and biting flies and phenology was observed regarding temperature and humidity records. Moreover, the potential dispersion of unfed or partially fed Tabanidae and Muscidae was assessed using challenging and innovative techniques such as *in vitro* feeding and mark-recapture experiments. 5396 biting flies (*Stomoxys calcitrans*, the stable fly) and 2104 tabanids of the genus *Haematopota* were collected in farm and on horses respectively. These flies and tabanids were colored using fluorescent dyes and half of them were partially fed with heparinized blood using an *in vitro* technique. They were released at increasing distances from bait horses surrounded by traps in order to assess the distance that unfed and partially fed vectors could fly and consequently the potential dispersion of the virus. This experiment was repeated several times without other animals in the vicinity and under appropriate weather conditions. These experiments demonstrated that the present

control measures based on a 200 meters buffer zone applied in case of introduction of the virus in Belgium are insufficient in order to prevent virus transmission.

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## CHARACTERIZATION OF SPONTANEOUS *RPOB* MUTATIONS CONFERRING RIFAMPICIN-RESISTANCE IN *BARTONELLA* ISOLATES

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The primary mechanism of bacterial resistance to rifampicin is associated with mutations in the RNA polymerase beta subunit (*rpoB*) gene. A single non-synonymous mutation in this gene can be sufficient to produce a rifampicin-resistant phenotype. Thus, this phenomenon has served as a model for the determination of single-locus mutation frequencies/rates in many bacteria including *Escherichia coli* and *Salmonella* species. The vector-borne *Bartonella* bacteria have been extensively characterized by the comparison of the sequences of their housekeeping genes (including the *rpoB*) and other conserved loci. Phylogenetic analyses have shown a vast genetic diversity within these loci, suggesting that these organisms may be undergoing an accelerated evolution. In the present study, we explored the occurrence of *rpoB* mutations conferring rifampicin resistance in five *Bartonella* spp., including the zoonotic *Bartonella henselae*, *B. grahamii* and *B. elizabethae* as well as two other *Bartonella* strains isolated from wild rodents and fleas. The minimal inhibitory concentrations (MICs) to rifampicin were determined by the use of Etest (BioMérieux, Marcy-l'Étoile, France). Rifampicin resistant mutants were isolated from the inhibition zone following each incubation trial. The MICs of the isolated resistant clones were determined by new Etest runs. Then, the DNA of the original and resistant clones was extracted and a fragment of the *rpoB* gene was PCR-amplified, sequenced and compared. The results showed that all original isolates (isogenic strains) were highly susceptible to rifampicin, with MICs ranging from 0.006-0.023 µg/ml. Interestingly, rifampicin resistant clones were frequently detected in most of the *Bartonella* spp. tested. One to four resistant colonies were observed within the inhibition zone of each Etest run. The MICs of the resistant clones increased from 4 to up to 5300 times the MICs of the isogenic susceptible strains. The phylogenetic analyses of the *rpoB* amplicons determined a single base mutation in each of the resistant clones. Mutations were located within the *rpoB* region known as “rifampicin resistant determinant region (RRDR)”,

demonstrating truly resistant genotypes and not tolerant-phenotypes. The mutations observed were non-synonymous, changing the genetic code for structurally different amino acids (e.g. His to Tyr; Gly to Cys). Moreover, a fitness cost of certain mutants was observed, limiting the growth of the clones in liquid medium. To conclude, this study indicates that *Bartonella* spp. may be undergoing high mutation rates which might contribute to the vast genetic diversity observed in *Bartonella* in the wild.

## BROAD-RANGE DETECTION OF TICK-BORNE PATHOGENS IN EASTERN ROMANIA

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In Europe, ticks are the most important vectors of pathogens that cause both human and animal diseases. So far, around 60 bacteria, 30 parasites and 100 viruses are registered as potential tick-borne pathogens and the list continues to expand. Numerous studies have indicated the role of ticks in the transmission of pathogens that have implications in human health, and their medical importance has long since been acknowledged. Also, several reports indicated ticks capacity to harbour two or more pathogens and transmit them simultaneously causing multiple infections, events that are not rare in humans. In Romania however, most surveys of ticks have concentrated on the identification of a limited number of bacterial and parasitic tick-transmitted pathogens with low concern for the identification of co-infections and detection of viruses in questing ticks. The objective of our study was to identify the most important tick-borne pathogens in questing ticks collected from forested and urban areas and evaluate their co-infection rates in Eastern Romania as a gateway into Europe. We analyzed 557 questing adult or nymph hard ticks belonging to three species (*Ixodes ricinus*, *Dermacentor reticulatus* and *Haemaphysalis punctata*). Briefly, DNA samples were assessed by PCR and quantitative PCR to determine the infection rates of *Borrelia* spp., *Rickettsia* spp., *Anaplasma phagocytophilum*, *Bartonella* spp. and *Candidatus Neorhlichia mikurensis*. We also screened RNA samples by RT-qPCR to identify the infection with tick-borne encephalitis virus and Eyach virus. The global prevalence of ticks infected with one or more pathogens was 45.9%. We found high prevalence rates of *Borrelia* spp. (29.6%), *Rickettsia* spp. (14.3%) and moderate to low infection rates for *C. N. mikurensis* (5.2%), *Bartonella* spp. (4.1%) and *Anaplasma phagocytophilum* (1.2%). After testing for tick-borne encephalitis virus and Eyach virus, we did not obtain positive samples. We found that 7.1% of ticks were co-infected with two pathogens and 0.7% had association of three pathogens. Our findings confirm that ticks

are important pathogen vectors in both forested and urbanized areas. We have generated detailed data on the occurrence of zoonotic pathogens, representing solid foundation for further studies examining the risk of human tick-borne diseases.



## ***IXODES RICINUS* AV422 SALIVA PROTEIN - POSSIBLE USAGE IN TICK BITE CONFIRMATION**

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Spreading of several tick species, which is recorded in recent years, increases the risk of tick bites and disease acquiring. Thanks to saliva functions, ticks attach and feed unnoticed and undisturbed by the host. Therefore, tick bite anamnestic data can lack. To confirm tick bite, a method of choice is detection of specific antibodies developed in host against injected tick saliva proteins. AV422 is a tick-specific protein which is injected in host as saliva component and performs crucial antihemostatic and anticomplement functions in early phase of tick feeding. We used *Ixodes ricinus*, one of the most important tick species in Europe in terms of medical significance, as model organism. Larvae of *I. ricinus* ticks were repeatedly fed on rats, in order to investigate whether recombinant *I. ricinus* AV422 protein (rIrAV422) could serve as antigen for detection of specific antibodies in sera of host animals as markers of tick bite. For purpose of comparison, we fed *Dermacentor reticulatus* larvae on second group of rats. rIrAV422 was expressed in bacterial expression system, using gene coding sequence from adult ticks. Detection of specific antibodies in rat sera was performed by Western blot. While negative control was nonreactive, sera of rats, both hosts for *I. ricinus* and *D. reticulatus* larvae, reacted with rIrAV422, pointing out solely tick bite as the cause of the reactivity, i.e. presence of specific antibodies and conserved antigenic characteristics of the given saliva protein across these two genera. Changes in saliva composition recorded between life stages of ticks, as well as during the course of blood meal uptake, reflect tick-host adaptations and tick evasion strategy of host immune defense mechanisms. Previous findings that demonstrated presence of AV422 or AV422-like proteins in saliva proteome of nymphs and

adult ticks of several tick species, taken together with tick-specificity of AV422 and cross-reactivity features described in this study, suggest broader usage as possible reliable indicator of exposure to different tick species. Beside clinical applications, tests based upon AV422 could be also usefull in epidemiological studies of tick-borne diseases.

This work was supported by a grant from the Ministry of Education and Science, Republic of Serbia (Project No. 173006).

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## WG 2

# Barcoding, molecular diagnosis and next generation sequencing

## POSTER PRESENTATIONS

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## INVESTIGATION OF TICK-BORNE VIRUSES AND BACTERIA IN *IXODES RICINUS* AND *IXODES FRONTALIS* SPECIMENS COLLECTED FROM VEGETATION, BIRDS, CATTLE AND DEER IN SPAIN, 2009-2014

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Several human diseases caused by tick-borne viruses have been reported in Europe. Nevertheless, little is known about their epidemiology in Spain. *Ixodes ricinus* is one of their main vectors and it is also responsible for transmission of bacterial diseases. Furthermore, birds play an important role in the epidemiology of tick-borne diseases as spreaders of infected ticks, including *I. ricinus* and also ornithophilic ticks such as *Ixodes frontalis*. The aim of this study was to investigate the presence of viruses (*Flavivirus*, *Phlebovirus*, *Coltivirus* and *Orbivirus*) and bacteria (*Anaplasmataceae*, *Rickettsia*, *Borrelia* and *Spiroplasma*) in *I. ricinus* ticks collected from vegetation, cattle, deer and birds in La Rioja (North of Spain) and neighbouring areas, as well as in *I. frontalis* and unclassified *Ixodes* spp. specimens removed from birds in this area. A total of 966 *I. ricinus* ticks collected from vegetation (n=541), cattle (n=229), deer (n=157) and birds (n=39), and 51 *I. frontalis* and 64 *Ixodes* spp. captured from birds were analysed. Ticks were pooled (n=182) and homogenised. The RNA was extracted, digested and reverse-transcribed using commercial kits. cDNA extracts were tested with the Applied Biosystems® Eukaryotic 18S rRNA endogenous control to confirm adequate RNA extraction and cDNA generation. Pan-*Flavivirus*, pan-*Phlebovirus*, pan-*Coltivirus* and pan-*Orbivirus* real time PCRs were performed. Moreover, conventional PCRs were carried out to screen for bacteria. No virus RNA was detected in the samples with the techniques used. Bacterial sequences were obtained with a high identity with validated sequences from GenBank of *Anaplasma phagocytophilum* (n=39), *Anaplasma* sp. (n=4), *Ehrlichia* sp. (n=1), *Candidatus Neoehrlichia mikurensis* (n=1), *Rickettsia helvetica* (n=10), *Rickettsia monacensis* (n=5), *Borrelia turdi* (n=3), *Borrelia valaisiana* (n=2), *Borrelia garinii* (n=2), *Borrelia afzelii* (n=1) and *Spiroplasma* sp. closely related to *Spiroplasma ixodetis* (n=2). Moreover, two

sequences showed 98% identity with *Candidatus* *Cryptoplasma californiense*, a novel, recently proposed member of the Anaplasmataceae. These results confirm the presence of several zoonotic tick-borne bacteria in Spain. The detection of a *Spiroplasma* sp. in ticks from Spain is reported for the first time.

The 'Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica' (SEIMC) awarded a grant to A.M.P.

## INSIGHTS INTO VIRAL DIVERSITY OF QUESTING TICKS IN EASTERN ROMANIA

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Worldwide, ticks are responsible for transmitting the highest number of pathogens compared to any other arthropod. Identification of bacterial tick-transmitted pathogens was the spotlight for most surveys carried out in Romania, with less concern for the viral agents vectorized by ticks. The objective of our study was to characterize the virome of questing ticks from Eastern Romania as a gateway into Europe, by using high-throughput sequencing (HTS). We included 557 questing adult or nymph ticks (534 *Ixodes ricinus*, 4 *Dermacentor reticulatus* and 19 *Haemaphysalis punctata*) randomly distributed in three pools, collected from three distinct areas in Eastern Romania. Analysis of HTS data uncovered sequences with similarity to viruses that associate to vertebrates, arthropods and plants. The number of total viral reads obtained after the assembly step was 21 368 211, 32 337 393 and 29 676 963 respectively for each pool. Subsequently, assembled viral contigs generated from each pool totalized 436 956, 1 052 524 and 272 492 contigs. Taxonomic assignation of contigs considered as distant to known vertebrate viruses, revealed homology to viral agents of *Bunyaviridae* family, each pool recording a number of 58, 243 and 11 contigs. Among them, contigs mapped to recently described viruses from *Nairovirus* genus- South Bay virus, and from *Phlebovirus* genus namely blacklegged tick phlebovirus-1 and blacklegged tick phlebovirus-2 (71% to 100% homology). Prevalence of viral sequences in ticks was screened by PCR from cDNA of individual ticks, using specific primers for the sequences found by HTS. South Bay virus-like cDNA was detected in three *Ixodes ricinus* (one nymph, a female and a male) with a global prevalence of 0.5%, while no ticks were positive for *Phlebovirus*-like sequences. Two sequences South Bay

virus-like were validated and proved to be highly homogeneous (99% identity), originating from ticks collected at the same site (male and female). After establishing the prevalence and distribution of South Bay virus-like in ticks, the following step will be the attempt to isolate this new viral agent by intracerebral inoculation of type I interferon receptor knock-out mice. Currently, new tick-borne viruses are being isolated from humans with febrile illness or death following a tick bite while many other have been described not to be related with human illness, leading to new questions in the field of tick-borne disease research: are those new viruses potential pathogens for humans, and more importantly, could these new viruses be linked to human or animal diseases.

## **WG 3**

# **Geospatial tools in vector research**

## **ORAL PRESENTATIONS**

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## SPATIAL DISTRIBUTION AND ABUNDANCE OF *CULICOIDES IMICOLA* AND *OBSOLETUS* GROUP IN EUROPE

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Biting midges (Diptera, Ceratopogonidae) of the genus *Culicoides* are important vectors of veterinary important pathogens causing large economic losses in many European countries. *Culicoides imicola* and the *Obsoletus* group are considered to be the main vectors of bluetongue virus in Europe that affects cattle and sheep. Spatial-temporal modelling of the vector distribution and abundance allow to identify areas under risk of virus transmission and can help to apply effective surveillance and control measurements. For this purpose, presence-absence and abundance data of *Culicoides* were collated from 1005 sites across 9 countries (Spain, France, Denmark, Poland, Switzerland, Austria, Poland, Sweden, Norway) between the years 2007 and 2013. The dataset included information on the vector species

abundance (number of specimens caught per night), GPS coordinates of each trap, start and end dates of trapping. A dataset of 120 environmental variables obtained from a Fourier transformed MODIS temporal series were used with the Random Forest machine learning algorithm in VecMap developed by AviaGIS to predict the overall species distribution and monthly abundance in Europe. For every month of the year, maps were generated to visualize the abundance of *C. imicola* and *Obsoletus* group in Europe and a distribution map for each of both showing the probability of occurrence. Although the variance was large, the predicted abundance values for each site had a positive correlation with the observed abundance.

Spatial variation was found in abundance for *C. imicola* and the *Obsoletus* group. For *C. imicola* the highest abundance was found in Spain as for the *Obsoletus* group the highest abundance was found in eastern France. Temporal variation was also observed with the highest abundance occurring during summer months and the lowest during winter months. We were able to model the spatial distribution in Europe for *C. imicola* and *Obsoletus* group in terms of abundance and suitability. The maps obtained showed a consistent pattern with the reported distribution for *C. imicola* and the *Obsoletus* group. The observed seasonal variation was also consistent with the reported by literature as *Culicoides* population dynamic depends on environmental factors such as temperature and rainfall. The outputs obtained here can be used as inputs for epidemiological models and can be helpful for determining areas of under risk of transmission.

## DIVERSITY AND SPATIAL DISTRIBUTION OF SAND FLIES (DIPTERA: PSYCHODIDAE) IN SERBIA – 3 YEARS OVERVIEW

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After several decades of neglecting, cross-section survey of sand fly distribution and diversity in Serbia was conducted in period 2013-2015. Surveys in 2013 were confined only to North part of the country – Vojvodina region, where cases of canine leishmaniasis started to emerge. In 2014 surveillance was extended to South-East Serbia (ex-endemic area of visceral leishmaniasis and phleboviruses) and in 2015 to West and South Serbia (no previous data about sand flies or leishmaniasis). During the three year period, 130 villages were surveyed. Sand flies were collected indoor and outdoor with standard CDC light traps, dry ice baited traps without light (NS2), BG Sentinel + CO<sub>2</sub> traps, sticky traps and aspirators. Collected samples were identified morphologically and molecularly by sequencing the cytochrome oxidase 1 mitochondrial gene (COI 1) or with Maldi-Tof method. In total, 341 specimens belonging to 8 species of genus *Phlebotomus* were sampled. Most abundant species were *P. neglectus* and *P. papatasi*, followed by *P. tobbi*, *P. perfiliewi*, *P. simici*, *P. syriacus*, *P. mascittii* and *P. alexandri*. Diversity of sand flies varied greatly depending on the country region. Fauna was the richest in South-East Serbia where 6 species were present. Extending to the North (4 species), West (4) or South (3), number of species decreased. The most abundant species on North is *P. papatasi*. Species of *P. major* complex (*P. neglectus* and *P. syriacus*) dominate in East and West Serbia, while predominant species in South is *P. tobbi*. Three species, *P. syriacus*, *P. mascittii* and *P. alexandri*, were recorded for the first time in Serbia. Despite the relatively low number of collected samples it seems that Serbia has high diversity of sand fly species.

This study was supported by the VectorNet project, and Ministry of education, science and technological development of Serbia project TR31084.

## THE DISTRIBUTION OF *Aedes albopictus* IN GREECE: CURRENT STATE AND FUTURE RISK MAPS

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Since the first record of *Aedes albopictus* between 2003-2004 in Corfu and Thesprotia the current invasive mosquito species (IMS) has been spreading in many other parts of Greece. As an effort to record the available data across the country distribution maps, at Regional Unit level, were developed. These maps were based on specimens and results of questionnaires, distributed to all companies engaged in mosquito management programs. According to the results the Asian tiger mosquito has been established in many Prefectures, such as the Peloponnese, the Ionian Islands, the Central Macedonia, Attica and Crete. Additionally, in the current study the future potentiality of *Aedes aegypti* spread and establishment over Greece and Italy is presented employing spatial distribution risk maps. Therefore, in the first step current Spatial Risk Databases for the establishment of IMS over Greece and Italy are developed using the meteorological parameters from the ECA&D project and changes in the climatic parameters in 2050's are estimated (using the NASA GISS GCM ModelE under the IPCC-A1B emissions scenarios). In the next step, the mesoscale meteorological model WRF is used, to simulate the changes in the meteorological fields caused by climate change in a finer grid size using dynamical regional downscaling. Finally, in the fourth step the estimated changes in the meteorological parameters from step three are combined with the observation data from step one in order to estimate the future level of the climatic parameters of interest. The spatial distribution maps presented that many areas, mainly in the southern parts of

Greece and Italy, are suitable for the establishment of the *Aedes aegypti*. As such, in the context of climate change, it is particularly important to strengthen the surveillance of IMS in areas at risk of entry or establishment and risk for disease transmission.

Funding: LIFE CONOPS project “Development & demonstration of management plans against - the climate change enhanced - invasive mosquitoes in S. Europe” (LIFE12 ENV/GR/000466).

## MICROCLIMATIC TEMPERATURE PLAY A VITAL ROLE FOR VECTOR BORNE DISEASE TRANSMISSION IN THE COOL SCANDINAVIAN CLIMATE

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Temperature is a key driver of vector-borne disease transmission. Approximately 15<sup>0</sup> C is a threshold temperature for Bluetongue virus development in *Culicoides* vector. Most of the mathematical models for virus development time in vectors use standard meteorological temperature instead of the temperature in the microclimatic environment actually surrounding the vectors. The objectives of this study were to quantify the difference between the meteorological and the microclimatic temperature of *Culicoides* habitats, to develop a model able to predict the microclimatic temperature of an area based on available parameters from meteorological institutes, and to compare the impact of microclimatic and meteorological temperature on bluetongue virus development and the potential number of infectious bites produced by a *Culicoides* vector in its lifetime. We recorded half-hourly temperature in four microclimatic habitats; dry meadow, wet meadow, hedges, and trees at Strødam in Denmark from May-October 2015 by setting temperature data loggers in triplicates and at different heights in each habitat. From the same area we also recorded meteorological temperature, solar radiation, wind speed, humidity, and precipitation using a portable meteorological weather station. We performed multiple linear regressions to predict the microclimatic temperature of different habitats based on the parameters collected from meteorological weather stations. Finally, we modelled the bluetongue virus development time and number of infectious bites produced by a *Culicoides* infected with bluetongue virus in an hourly model. Compared to meteorological temperature, microclimatic temperature had more hours with >15<sup>0</sup>C in May to October 2015. Compared to meteorological temperature, microclimatic temperatures of dry meadow showed faster bluetongue virus development in *Culicoides* (median: 13 vs 18 days) and showed a higher number of infectious bites (median: 0.83 vs. 0.22 bites/infected *Culicoides*). In the multiple linear regression analysis, the

microclimatic temperature in different habitats was expressed as a function of meteorological temperature, solar radiation, wind-speed, precipitation, humidity, types of habitats, heights of loggers, months, and time of the day with an  $R^2$  value of above 0.80. Temperature is much higher in the microclimatic habitats; therefore virus development time in the vectors is much shorter than previously anticipated. The microclimatic temperature could explain why the relative cold Scandinavian countries experience *Culicoides* borne diseases in late autumn. The microclimatic models will allow us to predict the microclimatic temperature in Denmark using Danish meteorological data and thereby perform risk assessment of mosquitoes and *Culicoides* borne diseases.

## ASIAN TIGER MOSQUITO (*Aedes albopictus*) IN SERBIA AND MONTENEGRO: WHETHER EXPECTED CLIMATE CHANGE WILL INCREASE SUITABILITY

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The Asian tiger mosquito, *Aedes albopictus*, is ranked as one of the world's 100 worst invasive species. It is a competent vector for a wide range of arboviruses like Chikungunya, Dengue, Rift Valley fever and Zika. The vector has become well established in Europe since its introduction at the end of the 20th century, and now, as a result of climate change, is expected to undergo northward spread in western and central Europe, as well as an upward spread to higher altitudes. This makes modeling regional climatic suitability for establishment of *Ae. albopictus* a pressing issue. Future suitability and subsequent seasonal activity of the vector was investigated using three mechanistic models with climatic parameters from the EBU-POM regional climate model. The multi-model approach allows for the assessment of variability in the expected distribution and the evaluation of variable importance. Environmental parameters considered in the models are annual and seasonal temperature, mean temperature of the coldest month, annual rainfall and photoperiod. The datasets were assembled in R-project and ArcGIS. So far we have developed suitability maps for past and future climatic suitability for mosquito establishment for three characteristic periods: 1971-2000, 2001-2030 and 2071-2100. The preliminary results show that after a slight drop in suitability for the first part of the century most of Serbia will become significantly more suitable for the establishment of *Ae. albopictus*. This is due to an expected rise in seasonal and annual temperature by the end of the century. A similar approach was applied to Montenegro. We compare results obtained and discuss possible transport routes. There are currently no existing studies that cover climatic suitability modeling for the Balkans on a regional level.



This allows for the incorporation of small-scale heterogeneity in vector modeling, which can aid in identifying possible future habitats and thus shoulder monitoring and vector control.

## THE POTENTIAL OF GEOSPATIAL TOOLS: ENVIRONMENTAL RISK ASSESSMENT OF TICK-BORNE DISEASES TRANSMISSION

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Tick-borne diseases (TBD) are a public health issue worldwide. Understanding the spatial variation in TBD risk is essential for disease management and prevention. However, risk results from the combination of both hazard, the strength of the zoonotic transmission cycle, and exposure, the intensity of contact between people and infected ticks. Often, a narrow focus on hazard or on exposure is adopted, with little consideration for spatial heterogeneities that can occur in both and that materialize in human-environment interactions. We propose to combine a geographic, spatially explicit perspective on these issues, assessing not only land cover but also land use and management, with a detailed epidemiological outlook. Each step between the occurrence of a tick bite and the diagnostic of a disease case is considered in its spatial context. TBD are infectious diseases transmitted by the bite of an infected tick, which constitutes the first step of the causal chain leading to human cases. We conducted a survey on the incidence of tick bites in scout camps. Scouts are exposed to tick bites during summer camps as they are generally organised in rural settings within the vicinity of forests. We show that landscape composition and configuration around camp sites impact the incidence of tick bites. While the significant variables were in line with the current literature, effects related to human exposure were also highlighted. After the bite and depending of a complex set of factors (such as the length of tick attachment), an infection may or may not develop in the human host. Therefore, a study was conducted to assess which environmental conditions can favour the presence of *Borrelia burgdorferi* (*Bb*) infection in a group professionally at risk: farmers and veterinarians. Serological results for 148 veterinarians and farmers who

participated to the study were analysed. Using binary logistic regression, joint effects of landscape composition and configuration, forest and wildlife management were examined.

If a person is infected, s/he can develop a symptomatic case of tick-borne disease, or not. Finally, we focused on the disease itself by studying hospitalized cases of Lyme disease from two hospitals in Belgium and assessing the individual level epidemiological and environmental risk factors for Lyme disease. These results contribute to build a clearer picture of the risk of TBD in Belgium. The use of diverse data sources (tick bites, serological data and hospitalized cases) will help clarify the impact of landscape factors on the risk of TBD.

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## WG 3

# Geospatial tools in vector research

## POSTER PRESENTATIONS

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**DIROFILARIA IMMITIS: THE SEROPREVALENCE IN KENNEL DOGS OF SOUTHERN ITALY**

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*Dirofilaria immitis* is increasingly reported in dogs and is responsible for a disease with overlapping endemic areas, especially in Europe: dirofilariosis. Several factors (e.g. increased disease awareness, better diagnostic tools, climatic changes, seasonal population dynamics and movements of animals) may play a role in the recent rise in reports of infection in the various countries of Europe. The aim of this study was to investigate the seroprevalence of *D. immitis* in dogs from 68 kennels of southern Italy. The survey was carried out in the Campania region of southern Italy (latitude = 39°59'15"- 41°30'25"; longitude = 13°45'25"- 15°48'23") which extends over an area of 13,590 km<sup>2</sup>. The region is mainly hilly and extends from 0 to 1890 m above sea level. The climate is Mediterranean with dry summers and rainy winters. The weather conditions are suitable for the development of *Dirofilaria* in the mosquito vectors. In the 68 kennels, 537 blood samples (from 5 to 10 per each kennel) were collected. In each kennel we selected the dogs that were hosted for 2 years at least. The samples were transported to the laboratory and centrifuged at 3000 rpm for 10 min to obtain sera, then stored at -20°C until testing for seroprevalence of *D.immitis* using DiroCHEK® ELISA (Synbiotics, San Diego, USA) according to the manufacturer's instructions (sensitivity = 85-100% and specificity = 100%) [25]. Antigens of *D. immitis* were detected in 24/537 (4.4%; 95% Confidence Interval = 3.0-6.7) dogs in 6 out of the 68 kennels (8.8%; 95% CI= 3.6-18.9). The present study indicates that *Dirofilaria immitis* is present in Campania region in symptomatic dogs as well as in asymptomatic ones. Therefore, regular parasitological surveillance, appropriate treatment strategies and high quality standard of hygiene are required to guarantee the health and welfare of kennel dogs.

## PREDICTING SPATIAL DISTRIBUTION OF PATHOGENS TRANSMITTED BY TICKS IN NORTHERN EUROPE

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Ticks are the most important vectors of human and animal infectious disease in Europe. Among the pathogens transmitted by ticks are *Borrelia* spp, *Anaplasma* ssp and *Babesia* genus, which cause the important diseases Lyme borreliosis, and the less frequent human anaplasmosis and human babesiosis. Analyzing the spatial distribution of ticks and the pathogens they transmit is a key tool to assess human risk for tick borne diseases. Pathogens found in ticks are related to the presence of suitable hosts which depend on environmental factors such as temperature and rainfall, as they determine the host's suitable environmental conditions. Therefore spatial distribution can be modelled using environmental data as predictors. The aim of this study is to evaluate if it is possible to model the abundance of tick-borne pathogens and map their distribution in five countries of Europe based on environmental satellite data. Pathogen presence was screened from 15925 ticks, collected in 13 sites of England, Sweden, Denmark, Netherlands and France, divided in 49 pools of 25 nymphs per site. The presence of pathogens was detected using real-time PCR system. For this study, we selected as a training set for modelling six species of Bacteria (*B. burgdorferi*, *B. garinii*, *B. miyamotoi*, *Anaplasma phagocytophilum*, *Candidatus N. mikurensis*) and two species of parasites: *Babesia divergens* and *Babesia venatorum* (sp. EU1). Using environmental data from Modis Fourier transformed satellite imagery (1 km resolution) and Worldclim dataset, we generated abundance and distribution maps based on Random Forest machine learning

technique. Vecmap software<sup>TM</sup> was used for this analysis. The performance of the model was assessed using a Jackknife validation procedure and the most important variables have been identified using the mean decrease in GINI index. Preliminary maps showing the distribution and environmental suitability have been obtained for all the pathogens included in the training dataset, except for *Babesia divergens* for which no model could be done due to its low prevalence. Two species out of seven species the model was significant. These species were *Candidatus N. mikurensis* and *B. garinii*. The most important variables included temperature, precipitation and vegetation related variables. We were able to generate preliminary abundance maps for tick-borne disease pathogens. Creating spatial models with the ability to predict the presence, abundance and distribution of pathogens, is an important tool in the assessment of many tick borne diseases in Europe as they can be used to generate risk maps for tick-borne disease.

## COMMUNICATING SPATIAL VARIATION IN TICK-BORNE PATHOGEN PREVALENCE THROUGH A WEBSITE BASED ON NATIONAL SURVEILLANCE DATA

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Ticks are important vectors of both human and animal infectious diseases in Europe. Several new tick-borne pathogens (TBPs), not previously known to occur in Denmark, have been discovered in ticks in recent years. Prevalence and spatial variation in risk of exposure of these new pathogens are poorly known by risk groups as well as veterinarians and general practitioners. We want to not only improve surveillance of TBPs, but also increase awareness to ensure early diagnosis and more effective prevention of these diseases. A large amount of spatial prevalence data is available from recent surveillance of ticks and TBPs in Denmark. We are using these data to develop a website to communicate quantitative information on ticks, pathogens and the risk of infection and illness. In the near future, the newly EU funded ScandTick Innovation project will conduct large scale mapping of all TBPs in southern Scandinavia. Via our website, we aim to provide the public with knowledge of the risks of tick bites. We stratified our sampling by dividing Denmark into five regions, each of which was divided into two sub-regions. Some sampling sites were selected based on annual visitor numbers while others were selected based on nature type. Twenty-four sites were successfully sampled for ticks. At each site we collected 225 questing nymphs by flagging, and we used the BioMark™ real-time PCR system for high-throughput microfluidic real-time PCR amplification. This method allowed us to screen for 37 TBPs and 4 different tick species in a single run. Initially the website will cover Denmark, and will be expanded to southern Scandinavia during the ScandTick Innovation project period. We discovered and determined the prevalence of 12 TBPs in *Ixodes ricinus* nymphs, two of which were not previously recorded in Denmark. Despite the small size of the country we found a very large geographical variation in the prevalence of important pathogens. Therefore, maps are an ideal form of communication of the risk. The website will be easily accessible from a smart phone, and will address the public, veterinarians and general practitioners. It will include short, easy-to-read



sections on spatial risk, prevention and control, tick identification, tick biology, TBPs, the diseases and accompanying symptoms they may cause. To increase interest, we will continuously update the website with new information and recent case stories.

## GEOGRAPHICAL DISTRIBUTION OF *BORRELIA BURGDORFERI* ANTIBODY POSITIVE CASES FROM THE EGE UNIVERSITY SCHOOL OF MEDICINE – PRELIMINARY STUDY

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In the last decades, climate and environmental changes causes increase of infectious diseases, such as tick-borne, in the world and in Turkey. Lyme is tick transmitted infection and *B.burgdorferi* IgM and IgG antibodies are significant to diagnosis of the disease. In the study, we performed spatial cluster analysis of positive IgM +IgG antibodies of *B. burgdorferi* cases who are obtained from Ege University Medical School patient database. The samples were tested for the presence of *B. burgdorferi* IgG and IgM antibodies with a commercial kit (Lyme screen II, Vidas BioMérieux Vitek, France). Total of 65 patients of 116 cases who were evaluated only in the metropolitan area of Izmir due to their adress data. 28 of the cases diagnosed with active antibodies encounter the limit, 20 were weakly positive and 17 positive. Moran's (I) autocorellation test were made to detwermine spatial clusters of the cases in distrctit scale of the metropolitan area. A significant clustering was detected in the distribution of the cases. I index value of total cases is 0.41, positive cases are 0.22, limit values are 0.24, and weakly positve cases are 0.45 (Positive spatial auto-correlation occurs when Moran's I is close to +1. This means values are clustered together). Map of z score was prepared to understand the distribution of clusters. The clusters are mainly detected in the northern part of Izmir (Karsiyaka, Bayrakli Bornova and Kemalpassa towns). The cluster pattern is epidemiologically significant. Therefore the spatial epidemiological analysis was planned to understand the reason of these clusters.

## WG 4

# Phylogenetics and phylogeography of vectors and vector-borne pathogens

## POSTER PRESENTATIONS

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## PREVALENCE AND PHYLOGENETIC ANALYSIS OF BORRELIA MIYAMOTOI RELAPSING – FEVER GROUP SPIROCHETE IN IXODES RICINUS AND IXODES PERSULCATUS TICKS IN LATVIA

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Latvia is an endemic region for tick-borne infections like Lyme disease and tick-borne encephalitis virus which are registered annually. *Ixodes ricinus* and *Ixodes persulcatus* is two tick species which play significant epidemiological role in transmission of these infections in Latvia. Until now autochthonous clinical cases of relapsing-fever borreliosis were not diagnosed in Latvia. In this study, we would like to investigate whether host-seeking of the *Ixodes ricinus* and *Ixodes persulcatus* ticks, are infected naturally with relapsing-fever group spirochetes. In the present study tick samples collected from the environment in 2005–2007 in Latvia were screened for the presence of all *Borrelia* species by PCR and sequence analysis of the 16S rRNA gene. In total 887 ticks (318 *I. ricinus* and 569 *I. persulcatus*) were analyzed. *Borrelia* was detected in 36.2 % of all tick samples with a significant prevalence in *I. persulcatus* ticks (43.23 % (246/569) in *I. persulcatus* ticks and 23.6 % (75/318) in *I. ricinus* ticks,  $P=0.0001$ ). Sequencing revealed that from relapsing-fever group borreliae were detected only *Borrelia miyamotoi* and its total prevalence in ticks was 2.3 % without statistically significant difference of prevalence between both tick species (2.8 % (16/569) in *I. persulcatus* ticks and 1.3 % (4/318) in *I. ricinus* ticks,  $P=0.1614$ ). Phylogenetic analysis of the 16S rRNA gene of *B. miyamotoi* shows that in Latvia are circulated two genotypes: Russia/Europe type transmitted by *I. ricinus* ticks, and Russia/Asia - transmitted by *I. persulcatus* ticks. According data from literature, Russia/Asian type is more pathogenic than Russia/Europe type. This is the first report of *B. miyamotoi* finding in ticks in Latvia. Our results suggest that both *I. ricinus* and *I. persulcatus* ticks play important roles in the

epidemiology of *B. miyamotoi* in Latvia. This fact raises our suspicion that relapsing-fever borreliosis is underdiagnosed diseases in Latvia.

This work was supported by a grant of the Riga Stradin's University, Nr. RSU ZP 02/2013.

## **WG 5**

# **Rare and emerging vector-borne pathogens**

## **ORAL PRESENTATIONS**

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## PRESENCE OF *HEPATOZOON CANIS* IN RED FOXES AND TICKS FROM SERBIA

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*Hepatozoon canis* is a worldwide spread apicomplexan parasite of domestic dogs and wild canids. Its lifecycle requires two hosts, intermediate vertebrate and final hematophagous invertebrate, while brown dog tick *Rhipicephalus sanguines* considered to be the most significant vector species. Transmission of parasite to canine host is enabled by ingestion of infected tick. The role of red foxes (*Vulpes vulpes*) as reservoir species in enzootic cycle of *H. canis* has been emphasized in several European countries. *H. canis* was detected in foxes from Spain, Portugal, Italy, Croatia, Hungary, Slovakia and Romania. Recently, molecular based studies reported sporadic presence of *H. canis* in dog and *Ixodes ricinus* ticks from dogs in Serbia. The aim of our research was to investigate presence of *H. canis* in red foxes and their ticks from several localities in Serbia. During the period from 2011 to 2013, bodies of hunted and roadkill animals were collected and inspected for presence of ticks. Bodies of 67 foxes were collected at eight localities (Veliko Gradište, Krnjevo, Braničevo, Zatonje, Boljevc, Surčin, Ogar and Bukovac). A total of 80 ticks, belonging to five ixodid species: (*I. ricinus*, *I. hexagonus*, *I. canisuga*, *Haemaphysalis concinna* and *Dermacentor reticulatus*), were collected in fur of 27 foxes. The most numerous species was *I. ricinus* (62.5%), followed by *I. canisuga* (16.25%), *I. hexagonus* (8.75%), *H. concinna* and *D. reticulatus* (6.25%) each. Total DNA was isolated from individual ticks and fox spleens, and analyzed for the presence of *Hepatozoon* spp. by a conventional PCR targeting 666 bp long 18S rDNA fragment. Representative positive samples were sequenced. *Hepatozoon* sp. DNA was detected in 49 out of 67 analyzed spleen samples (73.13%) and 5 out of 80 tick samples, 3 *I. ricinus* and 2 *I. canisuga* ticks. Positive ticks parasitized three animals, which were also *Hepatozoon*- positive. Sequence analysis showed 99-100% identity with previously published *H. canis* sequences from GenBank. Though presence of *R. sanguineus* wasn't recorded on analyzed foxes, all animals were captured

within the known distribution range of that tick species in Serbia. High prevalence of *H. canis* detected in foxes confirms existence of sylvatic cycle in the studied area, indicates importance of this carnivore species as reservoir and points to it as possible health hazard to the domestic dog population.

This study was financially supported by project grant 01173006 (Ministry of Education, Science and Technological Development of the Republic of Serbia).



## PHENOLOGY AND SANDFLY ABUNDANCE IN *LEISHMANIA INFANTUM* ENDEMIC PERI-URBAN ECOSYSTEMS IN SOUTHEAST SPAIN

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Phlebotomine sandflies (Diptera: Psychodidae) are small blood-feeding insects of great medical and veterinary significance as vectors of *Leishmania infantum*, which are endemic in the Mediterranean basin, including southeast Spain. Of 12 sandfly species recorded in Spain, only two *Phlebotomus* (*Larroussius*) *ariasii* and *P. (L.) perniciosus*, are proven vectors of *L. infantum*. The incidence of Leishmaniosis is particularly high in peri-urban residential areas; however, there is no information on vector abundance and distribution in these areas. Here we report sandfly species abundance in the external environment (garden area) of 11 detached homes in Murcia (southeast Spain) in May-July and September-October 2013 and 2014 and the relationship between abundance and the microenvironment surrounding the traps. Eight hundred castor oil sticky traps (totaling 49.8 m<sup>2</sup>) were placed and sandflies were speciated morphologically. A total of 1298 sandflies were collected including 47% females and 53% males. Abundance peaked in July and was lowest in May and October. It varied significantly between and within houses and was greatest in traps located near walls and surrounded by ample vegetation. The most abundance specie was *Sergentomyia minuta* (67%) followed by *P. perniciosus* (19%), *P. papatasi* (1%), and *P. sergenti* (<1%). Moreover, 12% of sandflies were damaged and could not be unequivocally speciated. Among them, some specimens resembled the rare *P. p. chabaudi* and this will be confirmed using barcoding speciation methods. Study results confirm the presence of *L. infantum* vectors in the close proximity of human habitats. The predominance of *S. minuta* however, is likely associated to the trap type used (sticky traps) and the microenvironment in which they were placed and the presence of reptiles, its natural blood feeding host.

## HIGH DIVERSITY OF ZOONOTIC BACTERIA IN TICKS COLLECTED FROM SMALL MAMMALS IN ROMANIA

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In Europe, ticks are medically the most important group of arthropods, associated with high health-care costs for most established tick-borne disease and the newly emerging diseases transmitted all over the continent. While their medical importance is acknowledged and most diseases have a long epidemiological history, the majority of tick-borne diseases show an emerging pattern. The vector capacity and transmitted pathogens are known and established for tick species commonly occurring on humans, or those which can be collected using dragging. However, our knowledge on the importance of tick species or developmental stages found only or mostly on small mammals is limited. While small mammals are very common in a wide range of habitats and are known reservoirs of a number of zoonotic diseases, their ticks (and their vectorial importance) are unknown in Romania. Here we present data on the zoonotic potential of ticks collected from small mammals (voles, mice and shrews) trapped in agricultural areas surrounding rural settlements in several regions of Romania. Ticks were collected from small mammals (n= 217, 10 host species, 16 regions) and identified according to morphological keys. Four species of ticks were detected and used for DNA extraction and analyzed for the presence of *Rickettsia* spp. and *Anaplasma* spp, using generic primers. We found 5 species of zoonotic *Rickettsia* (*R. helvetica*, *R. japonica*, *R. monacensis*, *R. mongolitimonae* and *R. slovaca*) and *Anaplasma phagocytophilum*. This is the first report on the presence of *R. japonica* and *R. mongolitimonae* (both identified in *Rhipicephalus sanguineus*) in Romania and highlights the importance of small mammals in the zoonotic cycle of these pathogenic bacteria.

## **GOLDEN JACKALS (*CANIS AUREUS*) IN ROMANIA: A POTENTIAL NATURAL RSERVOIR OF *DIROFILARIA* SPP.**

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Carnivores in Europe may be infected by several species of filarioids (Spirurida, Onchocercidae). Of these, the most important are considered mosquito-borne *Dirofilaria immitis*, agent of heartworm disease and *D. repens*, which parasitizes in the subcutaneous tissues and is commonly zoonotic. Domestic dogs are the typical definitive hosts, but patent infections may occur also in other species of carnivores. The invasive nature and rapid spreading of the golden jackals (*Canis aureus*) throughout Europe raises questions regarding the involvement of this species in the life cycle of pathogens in its newly colonized territories. Between January 2014 and May 2015, 54 golden jackals originating from 18 locations from Romania were examined for the presence of adult filarioid nematodes by full necropsy. Clotted blood samples were collected from the heart of each animal and screened for the presence of 3 species of microfilariae that are commonly found in Romania (*D. immitis*, *D. repens*, *Acanthocheilonema reconditum*) by multiplex PCR. Overall, 30 nematodes morphologically identified as *D. immitis* were found in 18.52% (n=10) of animals, originating from the southern part of Romania. No *D. repens* or *A. reconditum* were found at necropsy. The molecular prevalence in blood samples from the same animals was 9.26% (n=5) for *D. immitis* and 1.85% (n=1) for *D. repens*. All samples were negative by PCR for *A. reconditum*. This

discrepancy may be the result of amicrofilaremic infections, as a result of prepatency or unisexual infections. The relatively high prevalence of patent *Dirofilaria* spp. infection, along with the increasing density and distribution range of golden jackal populations indicate their potential role in the diffusion of these zoonotic parasites and in the maintenance of natural disease foci.

## **THELAZIA CALLIPAEDA IN EASTERN EUROPE**

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*Thelazia callipaeda* (Spirurida, Thelaziidae) is a parasitic nematode of the eye of domestic and wild carnivores, lagomorphs and humans. This parasite is vectored in Europe by males of *Phortica variegata* (Diptera, Drosophilidae, Steganinae), a drosophilid displaying zoophilic behaviour, which feed on ocular secretions. *Thelazia callipaeda* was for long time known as the “oriental eyeworm”, for its original identification in Far Eastern Countries (e.g. China, Japan and Thailand). Since 1989, this nematode has been detected in many other European countries including Italy, France, Spain, Portugal, Switzerland, Germany and Greece, as agent of ocular infection of animals and humans. However, data on the occurrence of this parasite in the Eastern European countries were not available until 2014. Over the past two years, a number of autochthonous cases of ocular thelaziosis from animals living in Romania, Croatia, Serbia, Bosnia and Herzegovina, were published in the scientific literature. In addition, the zoonotic potential of this parasite was further confirmed by the report of human cases of thelaziosis from a 36-year-old and an 82-year-old patients living in Serbia and Croatia, respectively. In this study, nine dogs were referred to the Department of Parasitology, Trakia University, in Bulgaria, for the presence of nematodes in the conjunctival sac. All parasites collected from the dogs' eye were morphologically identified and molecularly characterized by sequencing of partial *cox1* gene. These new clinical cases represent further reports of

eyeworm infection by *T. callipaeda* from dogs living in Bulgaria. Finally, since human thelaziosis occurs in areas where the infection is prevalent in domestic and wild canids, felids and lagomorphs, a One Health approach is strongly advocated to tackle this vector-borne zoonosis.

## VECTOR COMPETENCE OF THE INVASIVE CONTAINER-BREEDING MOSQUITO *Aedes japonicus* FOR *Dirofilaria* spp.

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Transmission of the mosquito-borne filarial nematodes *Dirofilaria immitis* and *D. repens* depends on the availability of microfilariaemic hosts, competent mosquito species and suitable temperatures for the development of infectious stages in the mosquito. Apart from vector competence, abundances and host preferences of the mosquitoes are critical factors for assessment of vector capacity. The development of both *D. immitis* and *D. repens* in the container-breeding invasive species *Ae. japonicus* was tested under standardised experimental laboratory conditions in a laboratory strain (originating from the U.S.) and field-collected individuals from Zurich, Switzerland. Mosquitoes were allowed to feed through artificial membranes on canine blood containing microfilariae (mf) of *D. immitis* or *D. repens* at a density of 3.000 mf/ml. Blood-fed mosquitoes were incubated at 27 °C and 85% relative humidity and dissected at pre-set time points under a stereomicroscope to observe developmental stages in the various body parts. Additionally, real-time PCRs were carried out to assess infection rates. Feeding, mortality and infection rates as well as resulting vector efficiency indices (VEI) were calculated. The results show vector competence of the field-collected *Ae. japonicus*: First L3 of *D. immitis* were observed on day 10 post infection (p.i.) and of *D. repens* on day 7 p.i. The calculated VEI was 77.5% for *D. repens* and 100% for *D. immitis*, rendering *Ae. japonicus* endemic in Switzerland a competent vector for both filarial species. In contrast, the laboratory strain of *Ae. japonicus* was refractory to the development of *D. immitis* (VEI 0%, i.e. no L3 stages developed), even though it was moderately susceptible to *D. repens* (VEI 27%). This emphasizes the importance of using local vector populations when aiming to make risk assessments. In further experiments, realistic fluctuating temperature regimes will

be used (e.g. 21°C on average with range 12-29°C; 27°C on average with range 17.5-35°C, reflecting Swiss summer time conditions), as well as differing microfilarial densities. This will enable to realistically assess the risk of local transmission of these vector-borne helminths of both veterinary and zoonotic concern.



## DIROFILARIASIS IN LITHUANIA

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*Dirofilaria* infections in Europe are caused by *Dirofilaria immitis* and *D.repens*. Transmitted by mosquitoes, parasites can infect wild and domestic canines, felines, and humans. Climatic changes and an increase in the movement of reservoirs have an impact on the spread of dirofilariasis into previously non-endemic areas. In Lithuania, six human cases of *D. repens* infection were recorded during 2011-2015. In present study blood samples of 2180 randomly selected dogs presented in small animal clinic during 2014-2015 were investigated for filarial parasites. In four of dogs the adult worms were removed by using surgery technique. The microfilariae were determined on the basis of their morphometrical characteristics and the Diff-quick staining technique applied to blood smears and using Modified Knott's test. For differentiation and accurate identification of the filarial species we also used PCR with pan-filarial primers that amplify fragments of ITS2 region of rDNA from six different filariae species and species specific primers for amplification of *COI* gene of *D. repens*. Microfilariae were detected in blood smears of 4 (1.6%) dogs. Adults worm were identified as *D. repens* based on the macroscopic and microscopic characteristics and PCR analysis. Sequence analysis confirmed *D. repens* in all PCR positive samples. Additionally, blood samples collected from 101 shelter dogs were investigated for filarial parasites using PCR. *D. repens* was detected in 17.8% (18/101) of tested dogs.

## AN OVERVIEW ON NON-INDIGENEOUS MOSQUITOES AND EMERGING MOSQUITO-BORNE DISEASES IN EASTERN AUSTRIA

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Invasive, non-indigenous species (NIS) are a threat to biodiversity and have repeatedly been recognized as one of the most important factors in the extinction or displacement of native species on a global scale. In addition, they cause significant economic and health damage. Under globalization and climate change conditions it is assumed that the problems caused by NIS will increase and result in a growing displacement pressure on indigenous species. Above and beyond their ecological impact, invasive mosquitoes are a source of worldwide concern because of their utmost importance as vectors of a wide range of parasitic and viral pathogens affecting both animals and humans. Understanding current distribution and possible future expansion of invasive and native mosquito species is essential to guide coherent policy vector control in articulation with biodiversity conservation as well as environmental and human and animal health. As part of the ERA-Net BiodivERsA - GC-INVAMOFECT project the Eastern Austrian mosquito inventory and mosquito-borne diseases were examined. Until now 47 mosquito species were documented in Austria of which four are non-indigenous (*Aedes albopictus*, *Ochlerotatus japonicus japonicus*, *Anopheles hyrcanus* and *Culiseta longiareolata*). Our findings indicate that *Oc. japonicus japonicus* is present in the southern parts of Burgenland but further research has to be done to examine the status of invasiveness in this

area. Furthermore *An. hyrcanus* can be found in all three provinces in Eastern Austria (Burgenland, Vienna and Lower Austria). Beside the increase in the number of mosquito species in Austria, also the number of MBDs increased (e.g. *Dirofilaria repens*). First autochthonous findings of *D. repens* in mosquitoes in Eastern Austria in 2012 indicate that this pathogen is present in the research area. We will present preliminary results of the Austrian part of the GC-INVAMOFECT project (Vienna, Lower Austria and Burgenland), but also collaboration work conducted within the frame of this project (e.g. use of blood-fed mosquitoes for pathogen screening; Direct PCR of filarioid helminths and mosquitoes).

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## ARE HEAD LICE POTENTIAL DISEASE VECTORS?

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Humans have been infested with lice since their origins. During their co-evolution with humans, *Pediculus* species have adapted to ecological niches such as the hair, and later, clothing. Molecular phylogeny has been able to cluster *Pediculus* species into different genotypes, each group being comprised of head lice, however clothing lice only belong to groups A/D alongside head lice. The similarity between head and clothing lice begs the question as to whether head lice might be vectors of pathogens? Clothing lice are established with epidemic spread of trench fever (caused by *Bartonella quintana*); louse-borne typhus (caused by *Rickettsia prowazekii*); and louse-borne relapsing fever (caused by *Borrelia recurrentis*). Samples assessed included head lice from UK 88 individuals (23 of which were assessed using the microbial multi-pathogen array using DNA and 25 assessed using RNA multi-pathogen viral array); head lice extracted DNA 2010 Ethiopia n=74; clothing lice extracted DNA 1990 n=23; clothing lice collected in 1990s and stored frozen in ethanol n=20. Real-time PCR was undertaken to confirm louse ecotypes. Cytochrome B sequencing was conducted to confirm louse genotypes. Pathogen screening utilised a high-throughput screening using a fluidic multi-pathogen array devised for detection of tick-borne bacteria and viral pathogens. Of the head lice collected from UK 51/71 were confirmed to be head lice. The remaining 23 samples failed to yield amplicons, possibly through poor sample quantity. A microbial array including Lyme and Relapsing Fever *Borreliae*; *Anaplasma* spp; *Ehrlichia* spp; *Neorickettsiae*; Spotted Fever group *Rickettsiae*; *Bartonella henselae*; *Bartonella quintana*; *Francisella tularensis*; *Coxiella burnetii*; *Babesia* spp; and *Thileria* spp. were included and assessed using extracted DNA. Use of independent species-specific PCR assays confirmed the above findings with the exception of *Babesia* spp, presumed to be a cross-reaction. Pathogens can be found in head lice; however their competence as disease vectors remains to be established.

## EFFECT OF HEAT TREATMENT ON ANTIGEN TESTING OF SERUM SAMPLES FROM STRAY DOGS NATURALLY EXPOSED TO *DIROFILARIA IMMITIS* AND *DIROFILARIA REPENS* IN ROMANIA

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The aim of the present study was to determine the effect of heat treatment on antigen testing of serum samples from stray dogs naturally exposed to *Dirofilaria immitis* and *Dirofilaria repens* in Romania. A total of 194 stray dogs were sampled from five shelters in four counties situated in the eastern region of Romania and screened for presence of heartworm antigen before and after heat treatment, using a commercial microtiter well-based assay (DiroCHECK®, Zoetis). Modified Knott tests (n=108) and molecular studies (n=24) were performed in order to confirm the results from the present study. *D. immitis* circulating antigens were found in 16 (8.2%) non heated samples and in 52 (26.8%) heated samples. Of the 108 dogs examined by Knott test, 24 dogs (22.2%) were positive for circulating mf. Subsequent PCR identification showed six dogs had *D. immitis* mf only, 12 dogs, had only *D. repens* mf, and 5 were positive for both. Fifty % of dogs with circulating *D. immitis* mf had positive antigen tests before and after heating, while the other 50% reverted to positive only after heat treatment. Sixty % of dogs with mixed *D. immitis*/*D. repens* infection were antigen positive before and after heating, while the other 40% converted to positive after heating. Antigen testing for *D. immitis* in the 12 dogs with only *D. repens* mf gave conflicting results. Only two dogs (16%) were antigen negative both before and after heat treatment. Six dogs (50%) became antigen positive after heating and four dogs (30%) were antigen positive both before and after heat treatment. These results indicate that heat treatment of samples before

testing may reveal the true prevalence of heartworm infection in dogs. Moreover heat treatment of serum from *D. repens*-infected dogs can reveal an occult infection with *D. immitis*.

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## ANOTHER ONE BITES THE BIRDS – CHARACTERIZATION OF MALARIA INFECTION IN PENGUIN COLONIES HOUSED IN ZOOS

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Avian malaria is one of the most important causes of mortality in penguins housed in zoological collections. Knowledge of prevalence in zoos and the control measures applied (diagnostic, treatment and prophylaxis) allows other zoos to increase their colonies protection. In order to evaluate malaria infection in penguin colonies and zoos prophylactic programs, an online survey was specifically designed during 2014 to gather this information from different zoological gardens from Europe, North America and Asia. Fifteen (37,5%) out of the forty zoos that answered, test their colonies for malaria, revealing a global prevalence of 12,5% (5 zoos) of institutions with infected penguins. Diagnostic techniques most currently used were optical microscopy (11), histopathology (10) and observation of clinical signs (9). Twelve zoos use combined techniques in diagnostic protocols. Mixed infections with other blood parasites were reported by one zoo. Significant differences were not presented in clinical and laboratorial signs presented, being lethargy (4) the most prevalent sign. Nine zoos (22,5%) use treatment protocols on detected cases. No significant differences were observed in the protocols being the standard ones for most institutions based on sulfadiazine and pyrimethamine, chloroquine and primaquine. Twenty two zoos (55%) use prophylaxis protocols, being primaquine the most common drug in eight zoos. Seventeen zoos (42,5%) reported that no preventive measures besides preventive therapeutic protocols are used, while reducing the number of potential water catchment containers in order to eliminate the mosquito breeding sites available, was the measure most commonly adopted in 13 zoos (32,5%). Four zoological gardens (18,2%) stated that the penguins showed side effects after

prophylactic protocol and to the authors' best knowledge, these are the first reports of anorexia and vomit on penguins when using sulfadiazine and pyrimethamine; anorexia when using primaquine and chloroquine and epileptic seizures, sunburns around the eyes and death when using pyrimethamine. This study shows that many zoological gardens do not have routine control programs for malaria in their penguin colonies. Dissemination of these results allows a better understanding of this problem, raising awareness and potentially inducing new perspectives on its control.

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## SERUM PROTEOMIC CHANGES IN DOGS WITH EHRLICHIOSIS

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The aim of this study was to investigate the changes in serum protein response in dogs with ehrlichiosis by a proteomic study. For this purpose, three different pools were made: one from serum of healthy dogs, one from serum of dogs positive to *Ehrlichia canis* (E. canis) antibody serology without any evident clinical sign at physical examination (group of subclinical ehrlichiosis) and one from dogs positive to E. canis antibody serology with at least two clinical signs at physical examination compatible with canine ehrlichiosis (group of clinical ehrlichiosis). All these dogs were negative to *Dirofilaria immitis* antigen, *Borrelia burgdorferi* antibody, *Anaplasma phagocytophilum*/*Anaplasma platys* antibody and *Leishmania infantum* antibody. Three replicates of 2D-PAGE of each pool were performed and the spots of interest (those which showed significant differences in samples between healthy dogs, dogs with subclinic and dogs with clinic ehrlichiosis) were selected for MS identification. In brief, first dimension was carried out by running serum proteins in immobilized pH gradient (IPG) strips of 17 cm length and with linear pH 3-10 gradient. For the second dimension, reduced and alkylated strips were subjected to SDS-PAGE in 12% polyacrylamide gradient gels of 180 mm × 200 mm × 1.5 mm. Gels for MS analyses were stained with Page Blue. Stained 2D gels were scanned in an ImageScanner II (GE Healthcare Europe GmbH) and evaluated using specific software (Image Master 2D Platinum 7.0, GE Healthcare Europe GmbH). Images of all animal samples were digitalized and aligned. The separation and analysis of the tryptic digests of the selected spots were performed with a HPLC-MS system consisting of an Agilent 1100 Series HPLC (Agilent Technologies, Santa Clara, CA, USA) equipped with a wellplate autosampler and a capillary pump, and connected to an Agilent Ion Trap XCT Plus Mass Spectrometer (Agilent

Technologies, Santa Clara, CA, USA) using an electrospray (ESI) interface. Experimental parameters for HPLC were set in Chemstation software (Agilent Technologies, Rev. B.01.03), while Ion Trap parameters were set in LC/MSD Trap Control Software (Bruker Daltonik, v5.3). The statistical class analysis allowed the detection of 6 spot matches that showed significant changes between the different groups of animals. The HPLC/MS allowed the identification and characterization of these 6 spots as retinol-binding protein 4, apolipoprotein A-I, haptoglobin-like protein, alpha-1-acid glycoprotein 1, alpha-1-antitrypsin isoform X1 and vitamin D-binding protein. These proteins indicate that a series of changes in inflammation, lipid metabolism, cellular iron ion haemostasis and vit-D metabolism occur in canine ehrlichiosis and that these changes differ between the clinic and subclinical forms.

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## INFECTION BY *BORRELIA BURGDORFERI* S.L. AND *RICKETTSIA* SPP. IN TICKS FROM LIZARDS IN THE TUSCAN APPENNINE, ITALY

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During the investigations on ticks and tick-borne pathogens (TBP) range expansion in the Northern Appenines, Italy, in May-August 2011-2013, we captured 107 lizards (*Podarcis muralis*). Captures were carried out in nine sites ranging from 800 to 1600m of altitude, specifically chosen to be an optimal habitat for lizards (good sun exposure and abundant refuges). Sixty-eight animals were found infested by *Ixodes ricinus* (145 larvae, 25 nymphs; infesting 45 lizards), *Haemaphysalis sulcata* (119 larvae, 107 nymphs; infesting 37 lizards) and *Haemaphysalis punctata* (2 larvae; infesting two lizards). Coinfestations by *I. ricinus* and *Haemaphysalis* spp. were observed in 14 animals. *Borrelia burgdorferi* s.l. was detected in 3.5% *I. ricinus* larvae (number of positives=5, 95%CI: 1.2-8.0) and in two nymphs (8.0%, 95%CI: 1.0-26). Together with the species-specific *Borrelia lusitaniae*, we identified *Borrelia garinii*, *Borrelia afzelii* and *Borrelia valaisiana*. *I. ricinus* were also infected by *Rickettsia* spp. (17.6% of larvae, 95%CI: 11.7-24.9; 12.0% of nymphs, 95%CI: 2.5-31.2), namely *Rickettsia monacensis* (41.9%), *Rickettsia helvetica* (16.1%) and *Rickettsia hoogstraalii*, a *Rickettsia felis*-like organism that we detected in a sample of *H. sulcata* nymphs as well. The two *H. punctata* larvae did not harbour any bacteria. Coinfection by *B. afzelii* and *R. monacensis* was observed in one *I. ricinus* larva. Our results confirm the involvement of lizards in the transmission cycle of TBP. The heterogeneity of *B. burgdorferi* genospecies mirrors previous findings in small rodents' and questing ticks in the area, and suggests that lizards may be involved in the maintenance of genospecies other than *B. lusitaniae*. Moreover, we detected three rickettsial species other than *R. slovaca*, that has a natural focus of transmission in our study area and is associated to *Dermacentor marginatus*. We had already detected *R. monacensis* in *I. ricinus* attached on small rodents, but not *R. helvetica*, which other authors report as associated to

lizards. *R. hoogstraalii* is probably an endosymbiont of *Haemaphysalis* ticks in Europe; our finding of this organism in *I. ricinus* may have implications in public health, since it could preclude secondary infection with other -pathogenic- rickettsiae.

## OCCURRENCE OF TICKS IN THE SUBCUTANEOUS TISSUE OF RED FOXES, *VULPES VULPES* IN CENTRAL AND EASTERN EUROPE

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An extensive survey of parasites in red foxes (*Vulpes vulpes*) was independently conducted in Romania, Czech Republic and Slovakia. Carcasses were examined by necropsy, and occasionally small, dark nodules apparently containing ticks were observed in several cases when skinning for filaria detection. Prevalence of these nodules was estimated. Histopathologic examination was performed using haematoxylin and eosin (HE) staining. Of 91 foxes from Czech Republic, 16 (17.6 %) were harboring ticks in the subcutaneous tissue. In the majority of cases, 1 to 3 nodules/fox were found, with a maximum of 31 nodules/fox. In Romania and Slovakia a single case only in each country presented subcutaneous ticks, with 10 and 7 nodules respectively, containing apparently non-decomposed ticks. All of them were partially engorged adults. The following species were identified by morphological keys: *Ixodes ricinus* (1 female in Romania), *I. hexagonus* (2 females and 1 male in Czech Republic) and *Dermacentor reticulatus* (1 female in Czech Republic). The histopathologic examination of the skin lesions revealed chronic granulomatous panniculitis with peripheral fibrosis and intralesional presence of the ticks. In the inner part of the tick was observed a mixture of cell debris, cholesterol crystal clefts, macrophages and neutrophils. The cuticle was surrounded by inflammatory reaction consisting of macrophages, epithelioid, giant and

polymorphonuclear cells. These findings suggest that the ticks actively penetrated the cutis rather than being surrounded by the host's dermal tissue overgrowth. No data are available regarding ticks localized in the subcutaneous tissue of any host. Given this, it is difficult to provide comparative data or develop hypothesis. Nevertheless, all ticks were dead and even otherwise, it is hard to assume that this location represents an evolutionary advantage as their detachment from the granuloma it is probably impossible.

## MOLECULAR SURVEY OF CANINE BABESIOSIS IN DOGS FROM IVORY COAST

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Canine babesiosis is a common and clinically significant tick-borne haemoprotozoan disease of dogs and wild canids, with a worldwide distribution. The infection is transmitted by ticks belonging to the genera *Dermacentor*, *Haemaphysalis* and *Rhipicephalus*. Based on cross-immunity, serological testing, vector specificity and molecular phylogeny, large canine *Babesia* were classified into three species, namely *B. canis*, *B. rossi* and *B. vogeli*, of which the former two are the main species causing canine babesiosis in Africa. *B. rossi* is responsible for severe disease that can be life-threatening and is the most prevalent species infecting dogs in Africa. The aim of the present study was to assess the occurrence of canine babesiosis in dogs from Ivory Coast. This study was performed in 8 localities from Ivory Coast. A total number of 128 domestic dogs were sampled. Blood samples were collected in April 2013 and stored in absolute ethanol until further processing. Genomic DNA extraction was performed and all samples were examined by polymerase chain reaction (PCR). Specific primers for *Babesia* spp. were used, according to literature. Thirty-three dogs were positive for piroplasms (25.78%, 95% IC: 18.46%-34.26%). The local prevalence varied between 4.35% and 33.33%. The highest prevalence was recorded in Taabo. No significant difference in prevalence was observed between the genders. PCR products were sequenced and interestingly, all samples showed a 99-100% similarity to strains of *B. canis*. This fact was quite surprising, as the most frequent species infecting African dogs is *B. rossi*. The present study is the first approach of canine babesiosis in Western Africa, demonstrating its importance in the region and providing new insights into the distribution of *B. canis*.

## ***BORRELIA PERSICA* IN *ORNITHODOROS* TICKS IN ISRAEL, A CROSS SECTIONAL STUDY**

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*Borrelia persica* is a bacterium that causes tick-borne relapsing fever in humans in Eurasia and is transmitted by the soft tick *Ornithodoros tholozani*. This tick shows indiscriminate host feeding and *B. persica* has been presumed to be transmitted transovarially making the tick itself a reservoir. The aim of this study was to evaluate soft tick populations in multiple sites in Israel, determine the presence of *B. persica*, the sources of blood meal and finally, the risk-factors that affect the prevalence of *B. persica* in the ticks. We applied a molecular approach for species-specific identification based on PCR and nucleotide sequence analysis. Twenty four sites were surveyed and a total of 51,472 ticks from 15 caves were collected during October 2014 to August 2015. DNA of 2,774 ticks (1,035 nymphs, 787 larvae, 523 adult males, 429 adult females) was analyzed. *Borrelia persica* was found in 72 ticks (2.6%) from 9 (60%) of the caves analyzed, representing a prevalence of 1-8%. Larvae had the lowest prevalence of *Borrelia* compared to females, males and nymphs, with only 3 positive individuals (0.04%, 3%, 4.4% and 3.2%, respectively,  $p < 0.001$ ). Blood meals were found in 1,396 tick samples. Blood of 25 species of vertebrates was found in the blood meal analysis, including porcupine (*Hystrix indica*), jackal (*Canis aureus*), fox (*Vulpes vulpes*), badger (*Meles meles*), hyrax (*Procavia capensis*) and human blood, which were among the most abundant. Of the 72 *B. persica*-infected ticks, 49 ticks were positive for blood meal (67%). The presence of *B. persica* was found significantly related to the presence of blood meal in the ticks ( $p = 0.003$ ). Moreover, fox and jackal blood meals were significantly more frequent when compared to the rest of the animal blood meal sources in infected ticks ( $p < 0.001$ ). A univariate analysis revealed positive association between presence of *B. persica* in ticks and high average temperature and precipitation. A negative association was found with elevation ( $p < 0.039$ ). There was also a positive correlation between % of *B. persica*, with number of species hosts and number of canids present in the blood meal ( $r = 0.761$   $P = 0.001$ ,  $r = 0.696$   $P < 0.003$ , respectively). These results suggest that reservoir animals, in particular jackals and foxes, may play an important role in the natural transmission of *B. persica*.



## HEMORRHAGIC FEVER WITH RENAL SYNDROME AND LEPTOSPIROSIS, POTENTIAL RODENT-BORNE INFECTIOUS DISEASES IN ALBANIA

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Small mammals (Rodentia and Insectivora) are important hosts/reservoirs of hantaviruses and *Leptospira* spp. These pathogen agents cause severe infectious diseases in humans. Our study aimed the identification and distribution of small mammals in the country to provide important controlling and preventing data and information for the Public Health Services, as well as the identification of habitats' structure or vegetation and finding out the prevalence of hantavirus and *Leptospira* spp. among them. This study was performed during a two years period from May 2006 to November 2007 in 29 out of 36 districts of the country. Killing spring traps were set in forests, upper border of the forests, human inhabited areas, shrubs, meadows, lawns clearings and were collected early next morning. Captured small mammals were coded and preserved in thermobox with iced slabs to be transported in the lab for further examinations. In total, 263 small mammals (248 individuals of rodents and 15 of them insectivores) were captured in 352 locations/stations of the 29 Albanian districts. Results from molecular methods showed that Dobrava-Belgrade virus (DOBV) were detected in 10 of 148 (6.75%) *Apodemus flavicollis* rodents, and 2 out of 148 (1.35%) of them were positive for leptospira DNA. One (0.67%) individual of *A. flavicollis* was simultaneously infected with Dob/Bel and *Leptospira* spp. in one individual. These cases were detected in the districts of Diber, Korce, Kolonje, Librazhd, Pogradec and Vlore, mostly in the border part with Greece and FYROM. This suggesting that there is increased risk for hemorrhagic fever with renal syndrome and leptospirosis in rural and forestry areas in Albania. Correlation between the distribution and the presence of DOBV infected rodents in the country with human hemorrhagic fever with renal syndrome cases from previous studies showed that there is a

positive relationship in the same region between the positive human HFRS and the DOBV in rodents. Molecular testing of small mammals' organs for any probable infectious is performed for the very first time in our country.

## **WG 5**

# **Rare and emerging vector-borne pathogens**

## **POSTER PRESENTATIONS**

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## SERUM ACUTE PHASE PROTEINS IN DOGS WITH SUB-CLINICAL AND CLINICAL EHRLICHIOSIS

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The aim of this study was to investigate the possible differences in serum acute phase protein concentrations between dogs with positive serology for ehrlichiosis with and without clinical signs. For this purpose, dogs positive to *Ehrlichia canis* (E. canis) antibody serology were divided in two groups: without any evident clinical sign at physical examination (n=7, group I subclinical) and with at least two clinical signs at physical examination compatible with canine ehrlichiosis (n=8, group II clinical). All these dogs were negative to *Dirofilaria immitis* antigen, *Borrelia burgdorferi* antibody, *Anaplasma phagocytophilum*/*Anaplasma platys* antibody and *Leishmania infantum*. In all dogs, hematological and serum biochemistry analysis (comprehensive profile, VetScan) were performed and serum levels of C-reactive protein (CRP), haptoglobin (Hp), ferritin, albumin and paraoxonase-1 (PON1) were determined. There were no statistical differences on WBC and neutrophil counts in both groups. Observed hematocrit ( $23 \pm 5\%$ ), MCV ( $58 \pm 2$  fL) and MCHC values ( $29 \pm 3$  g/dL) in group II was lower ( $P < 0.01 - 0.001$ ) than those of group I ( $41 \pm 7\%$ ,  $64 \pm 4$  fL, and  $32 \pm 1$  g/dL, respectively). Marked thrombocytopenia ( $52 \pm 44 \times 10^3/\mu\text{L}$ ) was determined ( $P < 0.01$ ) in group II, but dogs in group I had a normal platelet count ( $254 \pm 140 \times 10^3/\mu\text{L}$ ). Serum ALT enzyme activity was higher ( $P > 0.05$ ) in group II ( $220 \pm 176$  IU/L) than in group I ( $41 \pm 16$  IU/L). Serum levels of renal injury markers (BUN and Cr) did not differ statistically between groups. Serum CRP and ferritin showed a significant increase ( $84 \pm 15$   $\mu\text{g/mL}$  and  $798 \pm 190$   $\mu\text{g/L}$ ,  $P < 0.001$ ) in dogs with clinical ehrlichiosis, compared to dogs with sub-clinicals ( $9 \pm 7$   $\mu\text{g/mL}$  and  $294 \pm 113$   $\mu\text{g/L}$ , respectively), whereas Hp did not show significant changes between groups ( $498 \pm 91$  mg/dL vs  $531 \pm 52$  mg/dL). Serum total protein, albumin levels

and PON1 activities were lower ( $P < 0.05 - 0.001$ ) in group II than in group I. The results indicate that in dogs with clinical ehrlichiosis there is an active inflammation with increases in CRP and ferritin and decreases in PON1 and albumin. On the other hand in ehrlichia positive dogs, the finding of a normal number of platelet and APPs in reference range would indicate the presence of the subclinical form.

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## MOLECULAR EPIDEMIOLOGY OF MOSQUITO-BORNE-DISEASES IN EASTERN AUSTRIA

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A range of factors (e.g. climate change, global trade and people/pet movement) have major impacts on the distribution of invertebrate vectors (e.g. mosquitoes), invasive pathogens (e.g. *Dirofilaria repens*) and their vertebrate hosts (humans, pets, livestock and wild animals). In recent years the permanent establishment of new competent vectors was facilitated, leading to an emergence and spread of vector-borne infections. Findings of *D. repens* and West Nile virus in mosquitoes in eastern Austria indicate a potential invasion of these pathogens, which might become of certain interest for public health and/or veterinary medicine in the next few years. Information about the distribution of mosquitoes and their potential vector competence, provide valuable information for scientists and public health officials. However, there is virtually no information about the pathogen load transmitted by mosquitoes in metropolitan Vienna but also in Lower Austria and Burgenland. Within this *ERA-Net BiodivERsA* project nearly 30,000 mosquitoes were captured in 2014 and 2015 at different sampling sites in Lower Austria, Burgenland and Vienna. Female mosquitoes were classified to species-level using morphological standard characteristics, pooled up to 50 individuals per date, species and sampling site and stored at -80°C. RNA and DNA were extracted and each pool was screened for the presence of flaviviral RNA, DNA of filarioid helminths, *Plasmodium*

spp. (avian malaria) and *Francisella tularensis* using molecular tools. DNA and RNA of various vector-borne pathogens were examined. We will present preliminary results of the pathogen screening and provide information on the distribution of those in examined areas during the investigation period of 2014 and 2015.

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## THE FIRST OCCURRENCE OF BLUE TONGUE VIRUS IN SLOVENIA

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On the 18th November 2015 blue tongue virus (BTV) was detected in blood of two cattle sampled for routine systematic surveillance for presence of blue tongue in Slovenia. Both cattle were clinically healthy. Never the less this was the first confirmed case of blue tongue virus infection in Slovenia. Characterisation of the isolate revealed that it was BTV serotype 4. At the day of virus confirmation Administration of the Republic of Slovenia for Food Safety, Veterinary Sector and Plant Protection was informed from Austrian authorities that they confirmed a case of BTV-4 just a little more than 24 km from Slovenian case. Slovenia implemented surveillance for blue tongue already in 2006, when an outbreak of BTV-8 occurred for the first time in Northern Europe. Active surveillance consists of entomological monitoring and serological screening of cattle and sheep. Entomological surveillance at 10 different sites all over Slovenia revealed that among competent vectors for BTV *Clulicoides pulicaris* and *obsoletus* complex are present in Slovenia. *Clulicoides imicola* has not been found in Slovenia so far. Corresponding to a monitoring program, total of 9.183 samples from cattle, sheep and goats were tested for the presence of BTV antibodies and BTV nucleic acid last year. After BTV-4 confirmation in Slovenia, zoning of Slovenia was implemented. Eastern half of Slovenia became zone with restrictions and western half remained without restrictions. Restriction of animal movement from zone with restrictions, disinsection of vehicles for animal movement during the season with active vectors and voluntary vaccination were implemented. The measures will stay in force for at least two seasons with active vectors from the last confirmation of BTV. All the measures implemented are in accordance with COMMISSION REGULATION (EC) No. 1266/2007.



## SELECTED VECTOR-BORNE ZONOSSES IN GRAZING CATTLE IN SLOVENIA – REVIEW OF EXISTING REPORTS AND CURRENT RESEARCH

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Granulocytic anaplasmosis (*Anaplasma phagocytophilum*), babesiosis (*Babesia divergens*) and Lyme borreliosis (*Borrelia burgdorferi* s.l.) are tick-borne diseases of cattle that are transmitted by the tick *Ixodes ricinus* in Europe. These infections appear in wide range of hosts. All of them are zoonoses and can be transmitted from cattle to humans via ticks. The disease course of mentioned illnesses is nonspecific and may be difficult to diagnose, except for babesiosis, without running specific laboratory tests. These infections may have impact on welfare and productivity in affected cattle. However, very few studies tackled vector borne diseases in animals in Slovenia so far. *A. phagocytophilum* was confirmed in game animals and in dogs. Clinical reports of *B. divergens* in cattle exist at the Veterinary faculty, while *B. burgdorferi* s. l. was confirmed serologically in dogs, horses and game animals, and Lyme spirochete has been confirmed in cattle in one study. The lack of relevant studies in animals in an area where the *Ixodes* ticks are abundant, where Lyme borreliosis is endemic in humans as well as occasional anaplasmosis, support the need to proceed with more systematic study of cattle vector-borne infections. Existence of cattle-specific strains of the above-mentioned causative agents is expected. Grazing cattle can serve as sentinels for human infections as they live close to human settlements, where outdoor activities of people take place as well. In the study, which will begin in May 2016 grazing cattle after turning out for one to two months will be sampled for presence of these three vector-borne pathogens in known hot spots of diseases in Slovenia. Detection of specific antibodies and pathogens is going to be performed in blood samples. Cattle will also be checked for ticks at predilection sites and collected ticks for the presence of pathogens. In addition, data (breed, age, pasturing history, location of pasture, presence of ticks) of cattle involved will be collected.

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## MIGRATORY BIRDS SUPPORTING INVASIONS OF *H. MARGINATUM* TICKS AND TICK-BORNE PATHOGENS INTO REPUBLIC OF MOLDOVA

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Migratory birds are often implicated in transporting ticks, which can carry pathogenic agents of several human and domestic animals diseases. By themselves ticks are not highly mobile, and the only ways to expand the habitat are hosts. The present study aimed to evaluate the prevalence and species of exotic ticks infesting wild migratory birds and to assess *Borrelia* and *Rickettsia* infection in the collected ticks. During spring we systematically caught and examined migratory birds coming to the Republic Moldova for tick presence. Migratory birds were caught in ornithological nets at natural reserve Prutul de Jos. All birds were identified to species level, and their sex. Birds were placed in bags made of thick fabric, right after birds were examined in the camp for the presence of ticks. After the examination, birds were released without being harmed. Tick species were detached from birds and stored individually in 70% alcohol. Total DNA was extracted using QIAamp DNA Mini Kit. We screened both ticks and for tick-borne pathogens, including *Rickettsia* species and *Borrelia*. Detection of *Borrelia burgdorferi* s.l. was performed by RT-PCR targeting *flaB* gene. *Rickettsia* spp. were detected by PCR targeting the *gltA* and *ompA* genes. On proprocession of two spring seasons (2014 and 2015), from the 203 avian specimens collected, 19% of birds (n=39) were infested by ticks (n=82). Ground-feeding birds were commonly infested. Four *H. m. marginatum* tick specimens were identified. Most collected ticks (93%) were *Ixodes ricinus* nymphs. Total of 28% of the ticks (n=23) were positive for infection with *B. burgdorferi* s.l. Additionally, two of *H. m. marginatum* ticks was positive for *Rickettsia* spp. *Hyalomma marginatum* ticks have not been registered on the territory of the Republic of Moldova since the 1980s. *H. m. marginatum* is a tick species usually occurring in relatively dry and warm regions of southern Europe, northern Africa and some parts of Asia. It is a vector of several disease agents of human relevance including Crimean-Congo hemorrhagic fever virus.

Reappearance of *Hyalomma* ticks should be taken seriously and draw further attention to the increasing problem of the import and spread of tropical vectors of disease to Eastern Europe.

## FOURTEEN YEAR SURVEILLANCE OF *R. TYPHI* IN AN ENDEMIC AREA IN GREECE

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Murine (endemic) typhus is caused by the flea-transmitted *Rickettsia typhi*. The classic cycle involves rats (*Rattus norvegicus* and *R. rattus*) as reservoirs and their fleas, particularly *Xenopsylla cheopis*, as vectors. Human infection usually occurs from contamination of the skin with rickettsiae-containing flea faeces or flea tissues, usually resulting in a moderate form of illness, with non-specific symptoms. The prefecture of Chania in western Crete (Greece) has been characterized as an endemic area of the pathogen. We present herein, the results of an ongoing surveillance protocol. Samples under suspicion for *R. typhi*, are routinely tested, by IFA and by Real-time PCR, at the Laboratory of Clinical Bacteriology, Parasitology, Zoonoses and Geographical Medicine (University of Crete), Reference Center for tick-borne diseases in Greece, in conjunction with the Regional Laboratory of Public Health of Crete. From 2011 and onwards, a more detailed history is been kept for each case, including clinical and epidemiological data. Since 2006, meteorological data are gathered from the Hellenic National Meteorological Service, together with data on the regions where the local Public Health Directorate has taken actions on rodent control. From January 2000 until November of 2014, 1650 samples had been tested of which 198 (infection rate 12%) were diagnosed as *R. typhi* infections. Of those, 91 were male and 107 were female. Non-typical symptoms were recorded, as expected. Diagnosis was set by IFA only in all cases; in 36 cases a 2<sup>nd</sup> sample was also received. All 66 whole blood samples received were tested negative by Real-time PCR. All patients received doxycycline. A somehow wavy pattern was observed, with the number of cases increasing and decreasing every 3-4 years. The number of cases was plotted against rainfall, humidity and temperature. The areas of residences of the cases and the regions where rodent control measures were taken were plotted in an attempt to identify high risk regions. Climatic conditions seem to influence their populations; fleas flourish in dry climatic

conditions with temperatures of 20–25 °C. Since *R. typhi* is directly associated with fleas, human cases may be indirectly associated with the number of fleas and of rats. We were not able to reveal a clear indirect association between climatic conditions and the number of human cases. Certainly more studies are needed on this field and in any case intensified control measures should always be a first line choice in order to minimize human cases in high risk areas.

## IMPACT OF CLIMATE ON SPOTTED FEVER GROUP RICKETTSIAE INFECTIONS IN HUMANS IN GREECE

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As from 2001, samples under suspicion for SFGR that are sent at the National Reference Center for tick-borne pathogens in Greece are checked either by IFA (using a slide that can test against *R. conorii*, *R. siberica mongolotimonae*, *R. slovaca*, *R. felis* and *R. massiliae* as individual antigens) and/or by Real-time PCR. Clinic-epidemiologic data of each patient and data on air temperature, wind direction, mean relative humidity and rainfall are gathered. We describe herein the first results of a large over-decade survey on SFGR in Greece. Data variables as time series (IBM SPSS v.21;  $p=0.05$ ) included the number of cases, temperature, humidity, rainfall level and wind direction. Descriptive analysis (to describe the basic data features), multiple linear regression analysis (to investigate if and how the variable number of cases depends on the quantitative variables) and the Fourier transform (for accessing their important frequencies) were applied. Phase portraits were produced for visualizing the underlying dynamics of the variables. To study the deterministic or non-deterministic nature of the variables, we utilized the correlation dimension ( $D_2$ ). Of the 6324 samples tested, 184 were positive for SFGR either by IFA or by PCR, mostly corresponding to *R. conorii* infections; four samples were tested positive for *R. slovaca*, seven for *R. siberica mongolotimonae*, eight for *R. felis*, one for *R. aeschlimanii* and one for *R. massiliae*. All patients had fever and a large distribution ( $>30$ ) of, largely, non-specific signs and symptoms. Cases peaked during 2005, followed by a continuous drop until 2008, and a rise thereafter. Generally, an increase in cases was observed in the presence of north, north-west/east winds. Cases seemed to increase a) during late summer and early autumn, b) when the temperature started to decrease, c) at the

beginning of slight rains and d) at decreased numbers of humidity. Despite the apparently deterministic nature of the complex dynamical system, cases' number did not significantly depend on any of the quantitative variables (absence of a meaningful linear regression model) or on seasonality. The change in climate together with altered human habits and interventions in the environment have led to a spread of tick species, hence to the continuous recording of human Rickettsial infections. Nowadays, there is a tendency of incorporating mathematical modeling and the observation of climate changes to export models that can act as early warning systems, although this approach, if applied alone, may not prove enough.

## **IN VITRO AND IN VIVO ASSESSMENT OF TRANSITION OF *FRANCISELLA* THROUGH BLOOD-BRAIN BARRIER**

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*Francisella* often invade multiple organs, such as skin, liver, lung and central nervous system. To invade various organs, *Francisella* has to cross cell barriers, mainly endothelial lining. We used two approaches to assess the translocation of *F. tularensis* subsp. *holarctica* strain LVS (live vaccine strain) through blood-brain barrier. For the *in vivo* invasion assay, Wistar rats were infected by *Francisella* by peritoneal road and animals were regularly checked to record the clinical symptoms. After 48 hrs after challenge, animals were euthanized, the cerebellum and cerebrum were collected and examined by histologic and immunohistochemical assays. For *in vitro* assay, the *in vitro* model of blood brain barrier was prepared and live and inactivated *Francisella* was added to luminal chamber and incubated for 24 hrs. The bacteria was subsequently isolated from the luminal and abluminal chamber and presence of bacteria was detected by PCR based of the *lpnA* gene. In the *in vivo* invasion assay, presence of LVS in the brain tissue of infected rat was detected. Clinical signs were observed 24 hrs after the challenge, when infected animals were found apathetic. Immunohistochemistry revealed *Francisella* in small aggregates of individuals tightly associated with blood vessel and also as a small foci observed in extracellular matrix. The *in vitro* assay shown translocation of live bacteria. This confirms that *F. tularensis* subsp. *holarctica* strain LVS readily translocate through blood brain barrier *in vivo* and *in vitro*. In presented work, we demonstrated that LVS is able to cross the brain microvasculature and infect the brain tissue *in vivo*, likewise it readily crosses BBB model *in vitro* in the absence of leukocytes. A complete understanding of



passage of *Francisella* through physiological barrier should contribute to the understanding of the pathogenic mechanism of *Francisella*.

## IMPORTED CUTANEOUS LEISHMANIASIS IN BULGARIA AND POTENTIAL RISK FOR INTRODUCTION IN NON-ENDEMIC COUNTRIES

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Cutaneous leishmaniasis is endemic in 87 countries worldwide – 20 in the New World and 67 in Old World. Between 1 and 1.5 million new cases of cutaneous leishmaniasis are registered annually in the Old World, with about 90% of them in Afghanistan, Algeria, Iran, Iraq, Saudi Arabia and Syria. Cutaneous leishmaniasis of the Old World is caused by five species of *Leishmania*: *L. infantum*, *L. tropica*, *L. major*, *L. aethiopica* and *L. donovani* with *Phlebotomus papatasi* and *Ph. sergenti* acting as major vectors. The disease is not endemic in Bulgaria and no indigenous cases have been registered to date. Between 2000 and 2015, twelve patients seeking medical help at the Department of Parasitology and Tropical Medicine in the National Centre of Infectious and Parasitic Diseases were diagnosed with cutaneous leishmaniasis. Four of them were Bulgarian citizens who have stayed in Afghanistan (n=1) and Sudan (n=3). The rest were Syrian citizens – three permanently residing in Bulgaria who have visited relatives in Syria and five refugees after 2010. As cutaneous leishmaniasis is not a notifiable disease in Bulgaria, the actual number of imported cases is unknown and probably higher than the registered. Clinical presentations of cutaneous leishmaniasis varied between single and multiple nodules and ulcers located on the face and upper limbs. All patients had history of local treatment of the ulcers with corticosteroids and antibiotics with no therapeutic effect. Material for parasitological diagnosis was obtained by fine- needle aspiration of the lesion edge. Microscopic examination of Giemsa-stained smears showed presence of amastigotes of *Leishmania sp.* All the patients were directed for cryotherapy with liquid nitrogen that effectively cured the lesions. Both historical and recent records show the presence of *Phlebotomus papatasi* and *Ph. sergenti* in Bulgaria, with the latter confined to the southern part of the country. With *Leishmania tropica* present in Greece, the Balkan region is proven vulnerable to import and transmission of anthroponotic cutaneous leishmaniasis. The

migration pressure in the region by people originating from leishmaniasis-endemic countries and long-term detention of such migrants in camps in the South of Bulgaria poses a threat for establishment of endemic foci and transmission by indigenous vectors.

## FIRST MOLECULAR DETECTION OF *BARTONELLA HENSELAE* AND *BARTONELLA CLARRIDGEIAE* IN CAT FLEAS FROM SERBIA

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Being associated with a broad range of serious human diseases *Bartonella* spp. are gaining importance within the last decades. Cat scratch disease is the most common zoonotic infection caused by *Bartonella henselae* and potentially *Bartonella clarridgeiae*. *Ctenocephalides felis* is the most common external parasite found worldwide on cats and dogs, and has been experimentally demonstrated as the vector for *B. henselae*. In order to determine the potential of fleas as vectors for *Bartonella* spp. infections of humans in Serbia, we analysed the *Bartonella* infection of fleas collected from cats. In May 2014, fleas were collected from cats at two localities in Serbia, Bačka Palanka and Kač. Prior to molecular diagnostics all fleas were identified morphologically using current taxonomic criteria. DNA was extracted from the fleas and tested by real-time PCR followed by HRM analysis, targeting the 16S-23S internal transcribed spacer (ITS). All positive samples were sequenced. A total of 16 fleas were collected and all were identified morphologically as *C. felis*. *Bartonella* DNA was detected in eight of the 16 fleas. Sequencing showed that they harbored two *Bartonella* spp. *B. clarridgeiae* (2/8) and *B. henselae* (6/8), both with 100% coverage and 100% identity with specific *Bartonella* species DNA, according to GenBank database reference sequences. We report the first molecular detection of *B. henselae* and *B. clarridgeiae* in cat fleas from Serbia. In the last few years, the zoonotic potential of *B. henselae* has been partially recognised in Serbia when scarce data on seroprevalence in cats as well the first report on human infection with this pathogen in Serbia have been published. Considering the small number of cat fleas included in our study, it is impossible to determine the accurate prevalence of *B. henselae* and *B. clarridgeiae*, but obtained data gives a new insight on epidemiology of flea-borne infections.

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## PHLEBOTOMINE SANDFLY ABUNDANCE IS STRONGLY AFFECTED BY TRAP POSITIONING HEIGHT

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Phlebotomine sandflies are diptera of considerable public and animal health importance. In Murcia Region (southeast Spain), where canine leishmaniasis is highly endemic, two of the eight recorded species, *Phlebotomus perniciosus* and *P. ariasi*, are proven vectors of *Leishmania infantum*. This study, aimed at determining the impact on sandfly abundance of the height at which sandfly traps are positioned taking into account on-site environmental factors such as wind speed, temperature and relative humidity. The study was carried out in an open-air dog kennel at Murcia Veterinary Faculty (38°00.501' N, 001°10.656' W; altitude 101 m) during 20 nights (8pm-8am) in September and October of 2015. We used castor oil impregnated A4 paper "sticky" traps 15 nights (3.75 m<sup>2</sup> sticky trap/night) and CO<sub>2</sub> (200 g dry ice)-baited CDC light traps 5 nights (3 traps/night). Traps were placed on a wire fence at three different heights above the ground: 0 cm, 75 cm and 150 cm. Temperature and relative humidity were recorded using a thermo-hygrometer (LogTag, CiK Solutions), and mean wind speed was recorded using an anemometer (Skywatch Eole, JDC Electronic). Sandflies were identified morphologically using taxonomic keys. A total of 282 sandflies were collected, 216 with sticky traps (57.6 sandflies/m<sup>2</sup>) and 66 sandflies with light traps. Species identified were *P. papatasi* (71%), *P. perniciosus* (18%) and *Sergentomyia minuta* (10%). The proportion of traps with sandflies and sandfly abundance were 95% and 241 sandflies, respectively, for traps situated at ground level, 75% and 35 sandflies, respectively, for traps at 75 cm above ground, and 25% and 6 sandflies, respectively, for traps 150 cm above ground ( $p < 0.05$ ). Random effects Poisson regression confirmed the strong association between sandfly counts and trap height and indicated that counts were also negatively associated to wind speed and were highest when relative humidity was lowest (34-56%) and temperature was moderate (23-24°C). Results highlight the relevance of trap positioning height and the need to monitor environmental factors when investigating sandfly abundance.

## **A POTENTIALLY NOVEL *CANDIDATUS NEOEHRlichia* SPECIES CLOSELY RELATED TO *CANDIDATUS NEOEHRlichia LOTORIS* IN RED FOXES FROM AUSTRIA AND CZECH REPUBLIC**

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The recently specified bacterial pathogen cluster *Candidatus* Neoehrlichia encompasses two closely related, tick transmitted candidate species, *Candidatus* Neoehrlichia mikurensis (CNM) and *Candidatus* Neoehrlichia lotoris (CNL). CNM showed to be relatively abundant in animals and humans from Central European countries, whereas CNL was solely found in raccoons from the USA. Spleen samples from a total of 164 red foxes from Austria and blood samples from 114 red foxes, 15 raccoons, 7 raccoon dogs, 4 beech martens, 3 European badgers, 4 European polecats, one European otter and one golden jackal from the Czech Republic were collected and examined for the presence of tick-borne bacteria of the family Anaplasmataceae by PCR and sequencing. Molecular analyses of 16S rRNA and *groEL* sequences revealed the presence of a new taxon, designated as *Candidatus* Neoehrlichia sp. (FU98), in spleen and

blood samples of two foxes from Austria (Vorarlberg) and the Czech Republic (Břeclav), respectively; this taxon is genetically closely related to CNL and clearly distinct from CNM.

The present study reports, for the first time, the occurrence of *Candidatus Neoehrlichia* sp. (FU98) in red foxes worldwide. More comprehensive studies on animals and ticks in other geographic regions are needed to explore the distribution, host range and possible vector species of this potentially new bacterial species. Moreover, future research on the isolation of this bacterium and transmission experiments are required, in order to elucidate its pathogenic features and its impact on animals and humans.



## SEASONAL AND SPATIAL VARIATIONS OF MOSQUITO COMMUNITIES IN EASTERN AUSTRIA DRIVEN BY ENVIRONMENTAL PARAMETERS

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Dispersal and establishment of indigenous and non-indigenous mosquito species as well as new emerging vector-borne diseases are supposed to be mainly driven by international trade and global change. Knowledge of mosquito species ecology as well as seasonal and spatial distribution patterns of mosquito communities is crucial to understand vector-pathogen dynamics in Europe. The mosquito species inventory of Austria consists until now of proven 46 species (including four alien species) belonging to seven genera (*Aedes*, *Anopheles*, *Coquilleidia*, *Culex*, *Culiseta*, *Ochlerotatus* and *Uranotaenia*). However, information on its genetic diversity, seasonal and spatial distribution patterns of communities and single species in Austria is insufficient. Mosquitoes were monitored at 27 permanent sampling sites, distributed across Eastern Austria (Vienna, Burgenland and Lower Austria) from April to October 2014. Female mosquitoes were sampled twice a month for 24 hours, using specific mosquito traps equipped with carbon dioxide as an attractant. Environmental parameters like humidity, air temperature, deciduous forest cover and breeding habitat availability for example were tested for significant effects on mosquito community composition using permutational multivariate analysis of variance (PERMANOVA) on dissimilarity matrices, based on raw mosquito data pooled per month and province. Parameters contributing significantly to the observed patterns were modelled in ordination space to estimate non-linear relationships. In this study we aim to update information on the current mosquito species inventory in Eastern Austria, the prevalence of mosquito-borne pathogens (e.g.,

filarioid helminths and avian malaria) and terrestrial environmental factors determining seasonal and spatial distribution patterns of mosquito communities. We will present preliminary results of the mosquito species inventory and discuss potential effects of habitat parameters on spatial and seasonal variations of mosquito communities in Vienna, Lower Austria and Burgenland.

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## THE CURRENT STATUS OF PHLEBOTOMINE SAND FLIES IN ALBANIA AND INCRIMINATION OF *PHLEBOTOMUS NEGLECTUS* (DIPTERA, PSYCHODIDAE) AS VECTOR OF *LEISHMANIA INFANTUM*

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Incidence of pediatric visceral leishmaniasis (VL) in Albania is higher than in other endemic countries of southern Europe, however the role of the endemic sand fly species as *Leishmania infantum* vectors has not been substantially addressed so far. In 2006 a country-wide collection of sand flies was performed in 14 sites targeted for the recent occurrence of VL cases. *Phlebotomus neglectus* was found the prevalent species (98.9%) among the *Larroussius* phlebotomines; 7/47 pools made of ~8 *P. neglectus* females each, have tested positive for *Leishmania* sp. genomic DNA. In the same year, sand flies were collected along with *Culicoides* trapped for bluetongue disease surveillance in 91 sites of southern Albania, selected by targeting farms with presence of large livestock regardless the occurrence of human VL. In 35/91 sites detected positive for sand flies, *Phlebotomus perfiliewi* was found to be prevalent (56.2%) among the *Larroussius* species. None of 7 *P. perfiliewi* female pools tested positive for *Leishmania* DNA. In 2011 *Leishmania* isolation and identification was attempted from dissected sand fly specimens collected in 4 sites characterized by high cumulative incidence of VL morbidity. Two positive specimens were found from a sample of 64 *P. neglectus* trapped in one site (3.1%). Parasites were successfully cultured from one specimen and were characterized as belonging to *Leishmania infantum* zymodeme MON-1, the same zymodeme identified in human and canine leishmaniasis cases from Albania. Altogether our studies indicate that *P. neglectus* is the main vector of leishmaniasis in Albania.